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RESPONSE OF THE CAT'S UTERUS TO THE HORMONES OF THE POSTERIOR PITUITARY LOBE

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(*Received* 27 *July* 1937)

The specific changes in response to the oxytocic factor of the posterior pituitary lobe which take place in the rabbit's uterus under the influence of the ovarian hormones have not hitherto been shown to occur in any other species. Thus in the mouse, the response of the uterus to oxytocin is greatly increased by the administration of œstrin, but this cannot be counteracted by progestin which in the rabbit inhibits the response [Siegmund, 1930]. The guinea-pig's uterus is even more different from that of the rabbit since neither œstrone nor progesterone appear to alter appreciably its response to the oxytocic hormone when the measurements are made in the intact animal.

The changes in response to oxytocin which take place in the course of pregnancy, have been investigated quantitatively in the rabbit, the mouse, and the human. Again the rabbit is exceptional in showing two distinct phases, one of absence of response to oxytocin in the early stages of pregnancy, and one of increased response in the later stages of pregnancy and at parturition, whilst in the mouse and human there is a high sensitivity at parturition but no complete loss of sensitivity in the early stages of gestation.

In the present investigation we have studied the changes brought about in the activity of the uterus of normal and spayed cats injected with œstrone, progesterone and testosterone and of animals at various stages of pregnancy. Our chief aim has been to determine whether any changes in response to oxytocin take place during pregnancy and whether they can be correlated with the action of the ovarian hormones in the same way as in the rabbit [Knaus, 1930; Robson, 1933].

In each experiment we have investigated the effect of vasopressin and of adrenaline on the uterus as well as that of oxytocin. The effect of adrenaline on the cat's uterus affords a useful indication of the presence of luteal activity [Van Dyke & Gustavson, 1929]. The action of vasopressin is of interest because as Robson [1936] and Morgan [1937] have shown, it can inhibit the uterine muscle under certain conditions and act as an antagonist to oxytocin. The reaction of the uterus to these substances has been tested both *in vitro* and *in vivo*.

Methods

Experiments were performed in thirty cats. The non-pregnant animals were as a rule spayed by the dorsal route and injected for periods ranging from 1 to 2 weeks with œstrone (2–5 times daily with 0·01 mg. crystalline œstrone in solution in oil), or with œstrone followed by progesterone. Progesterone was given over a period of 4 days, the total dose being 6 mg. Daily doses of 2·0 mg. of testosterone were given over a period of 4–5 days, but this was not preceded by the injection of œstrone.

The experiments were done under chloralose anæsthesia. The animals were eviscerated (the stomach, intestine and spleen being removed), and one horn of the uterus was removed and cut in two, and the two strips were suspended in oxygenated Ringer-Locke solution in 100 c.c. containers for duplicate *in vitro* determinations. The contractions of the longitudinal muscle of the other horn were registered by means of a Cushny myocardiograph, which made it possible to obtain a record of the uterine movements without interfering with the natural position of the organ. The apparatus consists of a fixed and a movable arm, the movements of the latter being transmitted by a thread to a horizontal lever writing on the kymograph. The two lever arms were connected to the muscle of the uterine horn at a distance of 3–4 cm. apart. The abdominal cavity was closed by a celluloid screen which had a slit through which the recording instrument passed.

In a few experiments on pregnant animals the contractions of the circular muscle were registered by means of a method similar to that previously used by one of the authors [Robson, 1936].

We are indebted for the purified samples of oxytocin and vasopressin to Dr White of Parke, Davis and Co. Messrs Schering and Co. kindly supplied the progesterone (Proluton) and Ciba Ltd. the testosterone.

Results

All the results are summarized in Table I. Several points require more detailed description.

The spontaneous activity of the cat's uterus *in situ* varies greatly in different animals. Three types of activity can be clearly distinguished *in vivo*. These may be described as (i) the "irregular type", consisting of comparatively small and irregular movements; (ii) the "frequent" type in which there is a succession of rapid contractions and the relaxation is less complete than in (iii) the "intermittent" type in which the contractions are of large amplitude and are separated by relatively long intervals of rest. This type of activity corresponds with that described by Newton [1933] in pregnant guinea-pigs. The three types of spontaneous activity are illustrated in Fig. 1.

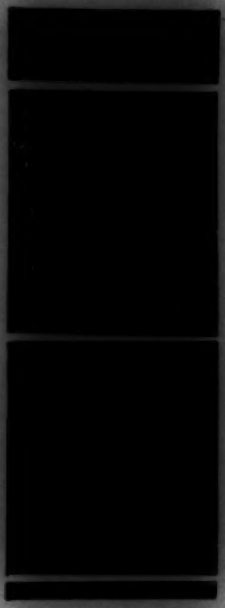

Fig. 1. Illustrating the three types of spontaneous activity observed *in vivo*. Upper tracing: irregular type. Spayed cat (C25). Middle tracing: frequent type. Spayed cat treated with œstrone and progesterone (C24). Lower tracing: intermittent type. Parturient cat (C14). Time intervals = min.

The "irregular" type of contraction is observed when the ovaries are not actively functional. It occurs in the spayed cats and in normal animals in the absence of either œstrus or pregnancy. The "frequent" type of contraction is observed in the uterus of animals treated with the ovarian hormones, œstrone, or œstrone followed by progesterone. It was also observed in one cat injected with transdehydroandrosterone, and in animals in the earlier stages of pregnancy. Lastly the slow "intermittent" type is found in the later stages of pregnancy, at parturition, and in the early puerperium.

There is thus a correlation between the type of contraction observed *in situ* and the functional state of the uterus. The *in vitro* experiments on the other hand show no such correlation. The shape and amplitude of the contractions observed *in vitro* have not the same characteristic differences which occur in the uterus *in situ*. The fre-

TABLE I. Showing the responses and spontaneous activities of the uteri of the experimental animals. Figures in brackets refer to response of circular muscle; all other figures to longitudinal muscle. Spontaneous activity: I = irregular; N = intermittent; F = frequent. Figures in italics denote inhibition. I.U. = international unit.

Condition	Injections	Response to oxytocin: *In vitro* I.U.	*In vitro* Av.	*In vivo* I.U.	*In vivo* Av.	Response to vasopressin: *In vitro* I.U.	*In vivo* I.U.	Response to adrenaline: *In vitro* γ	*In vivo* γ	Spontaneous activity: *In vitro*	*In vivo*
Spayed 15 days	Nil	0·05	0·06	0·5	0·23	>0·4	>0·5	>20	*30*	+ +	I
Spayed 14 days	,,	0·1		0·1		>0·5	>0·5	*20*	*10*	+ +	I
Dioestrous	,,	0·02		0·1		0·3	*0·3*	*10*	*20*	+	I
,,	Œstrone	>0·2	< 0·9	>0·1	0·27	>1·0	—	*100*	—	+ +	F
,,	,,	1·0		1·0		>1·0	*0·5*	—	*<50*	+	F
,,	,,	>1·0		0·2		>1·0	*0·02*	—	*10*	+ +	F
,,	,,	1·0		0·1		—	*0·1*	*100*	—	0	F
Spayed	,,	>1·0		0·2		>0·6	*<0·1*	—	*3*	0	F
,,	,,	>1·0		0·05		>1·0	*0·2*	—	*10*	+ +	F
Pseudo-pregnant	,,	0·02		0·02		0·1	—	*20*	*10*	0	F
Spayed	Œstrone and progesterone	0·05		—		>0·6	—	10	10	+	F
,,	,,	0·03	0·06	<0·05	0·06	0·1	>0·2	10	10	+ +	IF
,,	,,	0·1		0·1		>0·5	*0·5*	10	—	0	F
,,	,,	0·05		0·03		>0·6	*0·1*	10	30	0	F
,,	Testosterone	0·01	0·008	0·05		—	*0·1*	10	10	0	F
,,	,,	0·005		0·1		—	0·1	*10*	*10*	+ +	I
,,	,,	0·01		—		—	—	*10*	—	+ +	I
,,	Transdehydroandrosterone	0·01		0·05		—	—	*10*	*10*	0	F
Pregnant:											
Fœtuses = 4 mm.	Nil	>1·0		0·05		—	*0·1*	10	10	0	F
,, = 8 mm.	,,	0·1		0·05		0·5	>0·2	*5*	10	0	F
,, = 18 mm.	,,	0·2		0·2		—	—	20	—	+ +	F
		(0·2)									
,, = 20 mm.	,,	—		<0·1		—	*0·1*	—	10	+	F
,, = 10 cm.	,,	0·02		—		—	—	30	—	+ +	FN
,, = 13 cm.	,,	0·01		0·05		—	—	*5*	*10–10*	0	N
Parturient	,,	0·02	0·4	0·01	0·013	>0·1	>0·2	20	*10*	0	N
				(0·01)							
,,	,,	0·1		0·02		—	>0·1	10	*10*	0	N
				(0·01)							
Day after parturition	,,	0·005		(0·005)		>0·05	>0·05	20	*20*	0	N
				0·01							
Puerperium, 1st week	,,	0·05		<0·01		>0·6	>0·1	50	*10*	0	N
,, 13 days	,,	0·1		0·05		>0·6	*0·05*	>40	*10*	0	FN
,, 20 days	,,	0·01		0·02		>0·2	—	>10	*10*	0	I

quency of the contractions has therefore been taken as the sole measure of spontaneous activity *in vitro*. In all the groups of animals included in Table I totally quiescent uteri were occasionally found. No correlation could be detected between the frequency of movements *in vivo* and *in vitro*. Thus in the spayed animals small irregular movements were seen *in vivo* when large and frequent spontaneous contractions occurred *in vitro*, whilst in the œstrone and in the progesterone treated animals an intensive spontaneous contractility *in vivo* was frequently associated with complete quiescence *in vitro*.

In conclusion it may be stated that the contractions observed *in vivo* give a good indication of the functional state of the uterus, whilst the spontaneous contractions in the isolated organ vary in an apparently arbitrary manner.

Response to oxytocin. The average minimal effective doses of oxytocin in the various groups of animals are shown in Table I. In cases where no effect was obtained the maximal ineffective dose was used in the calculation of the averages.

The response of the uterus to oxytocin increases during pregnancy. Both *in vivo* and *in vitro* there is a maximum sensitivity towards the end of term and in the early puerperium.

Striking results were obtained *in vitro* in the hormone-treated animals. The uteri of œstrone-treated cats were practically insensitive to oxytocin *in vitro*, whilst after testosterone the response *in vitro* was of the same order as that obtained in parturient animals. The average difference in response between the œstrone and the testosterone groups is approximately a hundredfold.

No consistent differences in response were obtained *in vivo* between cats injected with œstrone, with œstrone followed by progesterone or with testosterone. Progesterone did not diminish the response to oxytocin either *in vivo* or *in vitro*.

Response to vasopressin. The characteristic action of vasopressin in cats injected with œstrone or œstrone and progesterone and in animals in the early stages of pregnancy consists of a prolonged inhibition of spontaneous movements and tone. This effect is, however, only obtained in the intact animal and in no experiment did vasopressin inhibit the uterus *in vitro*. The inhibitory effect of vasopressin on the uterus *in situ* persists after suprarenalectomy. Illustrations of the effects of vasopressin *in vivo* are shown in the next paper.

No vasopressin inhibition was obtained in the spayed animals and cats in the later stages of pregnancy. Inhibition by vasopressin seemed to be associated with the "frequent" type of contractions.

Occasionally vasopressin caused a small contraction of the uterus *in vitro*, and we are not satisfied that this effect may not have been due to some oxytocic substance present even in the purified samples of pitressin.

Response to adrenaline. Adrenaline caused a contraction of the uterus *in situ* in the progesterone-treated cats and during pregnancy. In one animal investigated during the latest stages of pregnancy a short contraction was followed by a prolonged inhibition, whilst during parturition and the puerperium adrenaline always caused a pure inhibition *in vivo*. Inhibition was also obtained in the spayed animals and marked inhibition was found in the œstrone-treated cats.

The results with testosterone were contradictory. In one of the spayed cats injected with testosterone (C31) there was a clear adrenaline reversal (contraction of the uterus), whilst in the other cases adrenaline inhibited the uterus. The histological examination of the endometrium revealed a progestational reaction in the uterus where the adrenaline reversal occurred (the animal had been castrated 4 days before the injections of testosterone were begun, and 6 days before the experiment). In the other two cats there was no progestational reaction of the endometrium.

The response to adrenaline *in vitro* agreed on the whole with the *in vivo* effects. The chief difference was seen in parturient animals in which a slight motor effect of adrenaline usually persisted *in vitro* but not *in vivo*.

Discussion

The uterus of the cat, like that of other species investigated, becomes more reactive to oxytocin in the course of pregnancy. It differs, however, from the uterus of both the rabbit and the mouse in revealing no increased response to oxytocin after treatment with œstrone. It further differs from the rabbit's uterus and is similar to that of the mouse, the guinea-pig and the dog in showing no loss of response to oxytocin after treatment with progesterone. It is improbable therefore that either œstrone or progesterone are solely responsible for the changes in response which take place in the cat's uterus during pregnancy. Testosterone causes sensitization of the uterine muscle to oxytocin, and it is of interest to note that Parkes [1937] has extracted a substance with androgenic properties from ovarian material.

The possibility that the alterations in the reactivity of the uterus which are obtained under various functional conditions may, in different species, be produced by different hormones has to be considered. Thus in the rabbit the sensitivity to oxytocin *in vitro* is increased by œstrone and decreased by testosterone, whilst in the cat the opposite is true.

There is no evidence at present that vasopressin plays any physiological role in the control of the uterine activity in pregnancy and parturition. Such a possibility cannot, however, be excluded, especially since, according to Starling & Verney [1925] the anti-diuretic principle, which appears to be identical with vasopressin [Fraser, 1937] is constantly secreted into the blood. The inhibition of uterine activity by vasopressin observed in the cat's uterus in the earlier stages of gestation, and also demonstrable in the rabbit's uterus under the influence of œstrone and progesterone [Robson, 1936], may possibly be a factor in maintaining the quiescence of the uterine muscle during pregnancy. The uterine quiescence which, as Reynolds [1932] has shown, follows the injection of gonadotropic hormone in the rabbit even in the absence of luteal function may also possibly be due to a vasopressin inhibition.

A direct comparison of effective doses in experiments *in vitro* and *in vivo* should be permissible, since drugs were added *in vitro* to 100 c.c. containers and injected intravenously into a blood volume of 100–150 c.c. Table I shows that in several instances the response of the uterus *in situ* differs from that of the organ suspended in Ringer-Locke solution. Thus, in cats treated with œstrone the response of the isolated uterus to oxytocin was greatly reduced as compared with that of the spayed animals, whilst the response of the organ *in situ* was not appreciably altered. A similar effect *in vitro* has previously been obtained in the bitch [Robson & Henderson, 1936].

These results in the cat and in the bitch stand in contradiction to the sensitizing effects produced by œstrin *in vitro* in certain other species, e.g. the rabbit and the mouse. The responses to vasopressin *in vitro* and *in vivo* will be discussed more fully in the next paper.

The discrepancies between the *in vivo* and *in vitro* results obtained in the present experiments suggest that in certain species it is necessary to investigate the spontaneous activity and the responses of the uterus in the intact animal in order to assess in a satisfactory manner the effects of hormones on the uterus.

SUMMARY

1. The action of oxytocin, vasopressin and adrenaline on non-pregnant, normal and spayed cats injected with œstrone, progesterone and testosterone and on pregnant, parturient and puerperal cats has been investigated.

2. Three types of spontaneous movements *in vivo* are described, associated with spaying, the action of the ovarian hormones and the later stages of pregnancy respectively.

3. The response to oxytocin of the uterus examined both *in vivo* and *in vitro* increases during pregnancy and is at a maximum at parturition and during the early puerperium.

4. Œstrone causes no increase in response and progesterone no decrease in response to oxytocin.

Œstrin decreases and testosterone increases the response to oxytocin *in vitro*.

5. Vasopressin inhibits the uterus of cats treated with the ovarian hormones *in situ*, but it has no effect on the uterus *in vitro*. Vasopressin produced no inhibition in spayed animals or in the later stages of pregnancy.

6. Adrenaline contracts the uterus of the progesterone treated, and of the pregnant cat. It relaxes the uterus of the spayed and œstrone-treated animal.

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EFFECT OF DRUGS ON THE BLOOD FLOW AND ACTIVITY OF THE UTERUS

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It has been shown in the preceding paper [Robson & Schild, 1937] that under certain conditions vasopressin inhibits the longitudinal muscle of the cat's uterus *in situ*, but that it has no effect on the isolated organ suspended in Ringer-Locke solution. The inhibition *in situ* was observed in spayed cats treated with the ovarian hormones, but not in untreated spayed cats or in animals in the later stages of pregnancy. The question arose whether this inhibition, obtained only in the intact animal and then under certain conditions, might be a consequence of the constriction of the blood vessels of the uterus produced by the pressor hormone. In this connexion the more general problem arose whether vasoconstriction and vasodilatation of the uterine blood vessels produced secondary changes in the state of contraction of the uterine muscle and also whether contraction of the muscle affects the blood flow through the organ.

The present research deals with these points. The blood flow through the uterus and the contractions of the organ were simultaneously registered and the effects of various drugs were investigated.

Methods

The experiments were done on cats under chloralose anæsthesia. A preliminary removal of the stomach, intestine and spleen was always performed.

The contractions of the longitudinal and the circular muscle of the uterus were registered by the methods previously described [Robson and Schild, 1937].

The blood flow through the uterus was estimated in the majority of experiments by means of a constant rate perfusion method. In others oncometers were used.

In the perfusion experiments the animal's own arterial blood was perfused at a constant rate by means of a small Dixon pump through one of the uterine arteries, coagulation being prevented by the intravenous injection of 0·1 g. chlorazol fast pink per Kg. body weight. The inflow pressure was registered by a Hg manometer and the contractions of the perfused horn were simultaneously registered. The venous return of the blood from the uterus was not interfered with. An illustration of the

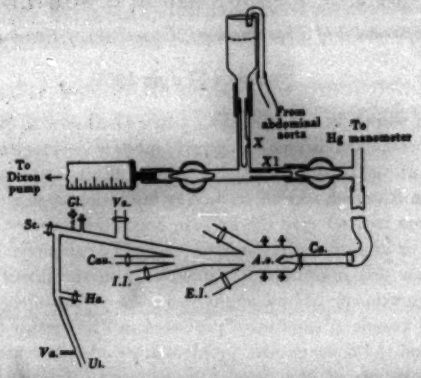

Fig. 1. Illustrating the method used in the perfusion experiments and the details of the dissection. The blood from the upper part of the abdominal aorta passes into the small reservoir and is then perfused by means of a Dixon pump through two small valves into the lower part of the aorta and thus into the uterine artery.

X, *X*1, valves; *Ca.* cannula; *A.o.* lower part of abdominal aorta; *E.I.* external iliac artery; *I.I.* internal iliac artery; *Cau.* caudal artery; *Ve.* vesical artery; *Gl.* gluteal artery (two are occasionally present); *Sc.* sciatic artery; *Ha.* haemorrhoidal artery; *Ut.* uterine artery; *Va.* small vaginal descending branch.

perfusion system is given in Fig. 1. The amount of blood delivered per min. by the pump was 1–5 c.c. The pump was fed by arterial blood drawn from the upper part of the abdominal aorta. In the majority of experiments the blood delivered by the pump was actually introduced into the lower part of the abdominal aorta and thence directed into the uterine arteries by tying off all the remaining branches of the aorta and of the iliac arteries. The dissection was usually controlled by a post-mortem examination. Fig. 1 gives a diagrammatic illustration of the blood vessels which were ligated in the course of the dissection.

In a second series of perfusion experiments one of the uterine horns was perfused directly through a cannula introduced into the uterine artery. This method was adopted in order to check the results obtained by the previous method with a technique which makes sure that all the blood perfused passes through the uterine circulation. The results obtained by the two methods were in agreement.

In the majority of experiments the uterine blood supply from the ovarian artery was arrested.

In the oncometer experiments, rigid wax oncometer, were used, moulded in each experiment to the shape of the uterus; the moulds were split into two and were joined together with grease. In the non-pregnant animal a whole uterine horn and in the pregnant animal a portion of the uterine horn containing a single fœtus was enclosed in the oncometer. When the uterus was long and easily mobilized it passed into the oncometer through a single opening, in the other cases two openings had to be provided for the passage of the uterus. The muscle was tied to the oncometer at the point of entry by means of a silver wire and the system was made air-tight with grease, care being taken not to occlude the blood supply. The volume changes inside the oncometer were transmitted by air to a sensitive paraffin piston volume recorder. Since in these experiments part of one horn was enclosed in the oncometer, the activity of the longitudinal muscle was simultaneously registered on the other horn. Changes in oncometer volume were interpreted as indicating alterations in the total blood content of the organ. This assumption appeared to be justified since the organ was well fixed within the oncometer and precautions were also taken to prevent uterine contents being moved through the uterine lumen in and out of the oncometer by closing the lumen at the point (or points) where the uterus entered the oncometer. The authors are aware that all oncometer experiments present certain technical drawbacks and the results are chiefly given as a confirmation by an entirely different method of the conclusions reached in the perfusion experiments.

Drugs were injected intravenously in the oncometer experiments, and directly into the arterial cannula (unless otherwise stated) in the perfusion experiments.

For details of the hormone preparations used reference should be made to the preceding paper [Robson & Schild, 1937].

Results

After the introduction of the arterial cannula and the initiation of perfusion, the perfusion pressure frequently rose. This could be partially prevented by maintaining a relatively low perfusion rate. Under such conditions the perfusion pressure was maintained quite level for one hour or more. Occasionally shallow waves were seen in the pressure record which appeared to be synchronous with the contractions of the uterine muscle. This was seen especially when the uterine artery was directly perfused (Fig. 3).

Fig. 2.

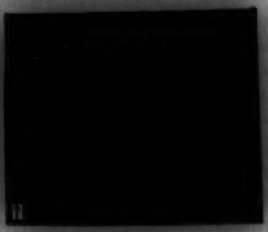

Fig. 3.

Fig. 2. Pregnant cat, 2·35 kg. Fœtuses 4 mm. in length. Upper tracing: contractions of the longitudinal muscle; contractions shown by upward movement. Lower tracing: oncometer volume; a decrease in the volume of oncometer contents is indicated by downward movement. 1·0 cm. of movement: 0·1 c.c.

A. At the arrow 0·1 unit vasopressin injected intravenously.

B. At the arrow 0·2 unit oxytocin injected intravenously. Time intervals: 1 min.

Fig. 3. Cat, 2·5 kg., 2 days after parturition. Upper tracing: contractions of longitudinal muscle. Lower tracing: perfusion pressure (direct perfusion of uterine artery). Note small alterations in perfusion pressure synchronous with the uterine contractions.

At the arrow 0·1 unit of oxytocin injected into perfusion fluid. Time intervals: 1 min.

Effects of oxytocin. The actions of oxytocin were investigated in both oncometer and perfusion experiments. In all experiments it was found that the doses of drug used (0·05–0·3 unit) were sufficient to produce contraction of the muscle, the cats weighing from 1·4 to 3·4 kg.

The oncometer experiments showed that muscular contraction was not associated with any change in the volume of the uterus. This is illustrated in Fig. 2B which shows the response of the uterus of an early pregnant animal to the intravenous injection of 0·2 unit of oxytocin.

In the perfusion experiments in which the blood flow through one horn and the contractions of the muscle of that horn were simultaneously registered, it was found that the addition to the perfusion fluid of a dose of oxytocin which produced a contraction had little or no effect on the blood flow.

Fig. 3 represents an experiment in which the uterus was perfused directly through the uterine artery. Oxytocin, whilst causing a powerful contraction of the uterus, produced only a small initial rise in blood pressure with some accentuation of the spontaneous waves present in the perfusion record. In the experiment shown in Fig. 4, performed on a spayed cat treated with œstrone, a slight rise was obtained after oxytocin. It is possible that these slight rises of perfusion pressure may be attributed to traces of vasopressin contained in the oxytocic preparation used. In one experiment performed in the later stages of pregnancy a small dose of oxytocin caused a contraction of the uterus without affecting the perfusion pressure.

Effects of vasopressin. The action of vasopressin on the uterine circulation was also investigated by the two methods described above.

The oncometer technique was employed in only a small number of experiments, involving two pregnant animals and one non-pregnant cat which was spayed and treated with œstrone. In these experiments the muscular contractions of the opposite horn were recorded at the same time. In two cats records of longitudinal muscle were taken, while in the third animal (pregnant) contractions of circular muscle were registered.

Six intravenous injections of vasopressin in doses of 0·05–0·2 unit were made in three experiments. In five cases there followed an immediate diminution in the volume of the contents of the oncometer and a decrease in the spontaneous fluctuations in volume. These changes were associated with an inhibition of the longitudinal and circular muscle, as recorded in the opposite horn. The effects are illustrated in Fig. 2A taken from an experiment on a pregnant animal (C19). In one experiment (C29) the early injection of vasopressin produced the usual effect, but later a second injection produced an increase in volume.

In the perfused organ vasopressin in doses of 0·05–0·1 unit injected into the perfusion fluid always caused vaso-constriction. The effect was more prolonged than that of adrenaline (Fig. 4) and it was obtained repeatedly in one and the same experiment without diminution in intensity. Such vaso-constriction effects following the injections of vasopressin were observed in spayed cats treated with œstrone or with

œstrone followed by progesterone, and also in pregnant cats (Fig. 5). Since vasopressin causes relaxation of the uterine muscle in non-pregnant animals treated with the ovarian hormones, and in early pregnancy but not in the later stages of gestation, it follows that vaso-constriction by vasopressin does not necessarily entail inhibition of the muscle.

In those preparations in which vasopressin affected both blood flow and muscular activity, it was noticed that the rise in perfusion pressure preceded muscular inhibition by a considerable period and that relaxation started only when vaso-constriction was well advanced.

Fig. 4. Spayed cat, 2·4 kg., treated with œstrone. Upper tracing: contractions of longitudinal muscle. Lower tracing: perfusion pressure. At first arrow: 0·25γ adrenaline added to perfusion fluid; at second arrow: 0·05 unit vasopressin; at third arrow: 0·1 unit oxytocin; and at fourth arrow: 0·5γ acetylcholine added to perfusion fluid. The fall in perfusion pressure seen before the addition of acetylcholine was produced by a decrease in the stroke volume. Time intervals: 1 min.

Effects of adrenaline. The response of the cat's uterus to adrenaline depends on the functional state of the corpus luteum. In pregnancy [Dale, 1906; Cushny, 1906], in pseudo-pregnancy [Van Dyke & Gustavson, 1929], and after treatment with progesterone [Robson & Schild, 1937], the cat's uterus is contracted by adrenaline. In the absence of the corpus luteum hormone adrenaline relaxes the uterus.

The effect of adrenaline on the uterine blood flow has been investigated in spayed cats treated with œstrone alone or with œstrone and progesterone and in pregnant animals. In every experiment adrenaline injected into the perfusion fluid or added to the reservoir in doses of 0·05–4·0γ produced a rise in the perfusion pressure.

There is always a characteristic time-lag between the onset of vaso-constriction and muscular inhibition, the muscle beginning to relax only

after vaso-constriction has reached, or almost reached, its maximum. On the other hand, if adrenaline produces a contraction of the uterus, e.g. in animals treated with progesterone, the augmentor effect on the longitudinal muscle sets in almost simultaneously with the vaso-constriction, and is frequently followed by a secondary inhibition. In some

Fig. 5.

Fig. 6.

Fig. 5. Pregnant cat, 2·5 kg. Upper tracing: contractions of longitudinal muscle. Lower tracing: perfusion pressure.

A. At arrow 0·5 unit vasopressin added to perfusion fluid.

B. At arrow 1·0γ adrenaline added to perfusion fluid. Time intervals: 1 min.

Fig. 6. Upper tracings: contractions of longitudinal muscle. Lower tracings: perfusion pressure.

A. Spayed cat, 2·4 kg., treated with œstrone and progesterone. At the arrow 4γ adrenaline added to reservoir.

B. Anœstrous cat, 1·7 kg. Direct perfusion of uterine artery. 0·3 mg. ergotoxine injected and 25 min. later, at the arrow, 4γ of adrenaline added to the perfusion fluid. Time intervals: 1 min.

cases, however, in the progesterone-treated animals, adrenaline produced vaso-constriction and contraction of the muscle without a trace of inhibition. An effect of this kind in a progesterone-treated animal is illustrated in Fig. 6A, where adrenaline was added to the perfusion reservoir, thus securing a slower and more prolonged action. Ergotoxine reverses the vaso-constrictor effect of adrenaline on the uterine circulation (Fig. 6B). The vaso-constriction and inhibition of the muscle brought

about by vasopressin are not altered by the previous administration of ergotoxine in doses which cause a reversal of the action of adrenaline.

Faradic stimulation of the hypogastric (presacral) nerve causes a rise of perfusion pressure and inhibition of muscular activity. After the administration of ergotoxine (0·3 mg.) its stimulation produces a fall in perfusion pressure.

Alterations in the oxygen supply. In view of the time-lag between the onset of vaso-constriction and of muscular inhibition it seemed possible that the inhibition of the muscle might be secondary to a decrease in oxygen supply brought about by the diminution in blood flow. The effects on the muscle of various factors causing alterations in the oxygen supply were therefore investigated. It was found that such experimental interferences as are associated with a decrease in the oxygen supply produce a decrease in the rate and amplitude of the rhythmic contractions.

The oxygen supply was altered by the following methods: (1) changes in the perfusion rate, and (2) perfusion with saline instead of blood. The effects of adding cyanide to the perfusion fluid were also determined.

The addition of 0·25 c.c. of *N*/10 NaCN to the perfusion reservoir was followed by a complete cessation of rhythmic motility and a fall in tone of the uterus; the perfusion pressure also fell. These effects were reversible and after some time perfusion pressure and uterine motility and tone returned to their former levels.

In some experiments an increase in the rate or stroke of the perfusion pump caused a rise in uterine motility.

When the uterus was perfused with non-oxygenated Ringer-Locke solution instead of blood there followed a fall in the perfusion pressure and a decrease in the uterine motility.

Discussion

No relation between the uterine blood flow and the motility and tone of the uterus has been demonstrated in these experiments. The various changes which may take place simultaneously in the muscle and the blood flow are shown in Table I.

A striking feature of these experiments has been the relatively small influence which muscular contraction exerts on blood flow through the uterus. Frequently indeed no effect at all was seen on the perfusion pressure during contraction of the uterus and only occasionally were the spontaneous contractions or the contractions produced by oxytocin reflected in the perfusion pressure record. Any diminution in blood flow

TABLE I. Effects of various agents on uterine blood flow and uterine muscle

Effect on muscle	Effect on blood vessels: Constriction	Dilatation	Little or no effect
Contraction	Adrenaline (after progesterone)	Acetylcholine	Oxytocin
Relaxation	Vasopressin Adrenaline	Cyanide Saline Adrenaline (after ergotoxine)	—
No effect	Vasopressin (late pregnancy)	—	—

during muscular contraction was of a much smaller order than the effects produced by the injection of vasopressin or adrenaline.

Since in most of these experiments the bulk of blood in the perfusion system was flowing through the body of the uterus—the collateral ovarian circulation was usually tied off and the upper vaginal circulation which derives from the uterine artery is comparatively insignificant—it followed that contraction of the muscle had no appreciable effect on the total volume of blood circulating through the uterus. The oncometer experiments, though on the whole technically less satisfactory, led to the same conclusion. Similar results were also obtained by Morgan [1937] in oncometer experiments performed on the rabbit's uterus. It is, however, conceivable that although the total volume of blood circulating through the uterus is not appreciably altered during the process of contraction, a shift may take place in the relative amounts of blood present in the endometrium and muscle respectively, during the period of contraction and relaxation. In the case of pregnant animals a possible shift of blood into the placenta and other uterine contents has also to be considered.

During relaxation of the uterus the blood flow may be either increased or decreased (Table I). No conclusion can, however, be drawn as to the effect of muscular relaxation *per se* on the blood supply to the uterus as drugs which we have used to produce relaxation all probably have an independent action on the blood vessels. In particular, the vaso-constrictor action of vasopressin and adrenaline would mask any possible increase in blood flow which might result from the muscular relaxation.

The experiments show that the inhibitor action of vasopressin on the uterus *in situ* is not due to a release of adrenaline, since (1) vasopressin produces inhibition after tying both suprarenals [Robson & Schild, 1937], (2) ergotoxine which reverses the vaso-constrictor action of adrenaline on the uterus does not abolish the vaso-constrictor effects

of vasopressin and (3) vasopressin produces a relaxation of the muscle in the cat treated with progesterone whereas adrenaline causes a contraction.

Whether the inhibition of the uterus is due to a direct action of vasopressin on the muscle or whether it is secondary to the decrease in blood flow cannot at present be stated with certainty. Since cyanide, saline and a decrease in the perfusion rate produce inhibition of the muscle, it would seem that the inhibition brought about by vasopressin might be due to a deficient supply or utilization of oxygen.

On the other hand, it is known that vasopressin can produce inhibition of the uterine muscle of the rabbit *in vitro* [Robson, 1932*a*, *b*] and also of the smooth muscle of the stomach *in vitro* [Robson, 1931]. These effects cannot be due to a vascular action and are presumably due to a direct action on the muscle. That the inhibitions obtained with vasopressin in the present experiments *in vivo* are also due to a direct action on the muscle and are not secondary to a rise in blood pressure is suggested by the following facts: (1) Vaso-constriction may be obtained together with muscular contraction, notably when adrenaline is acting on the uterus of the cat treated with progesterone. (2) In pregnant animals showing the "intermittent" type of contraction vasopressin produces vaso-constriction, yet there is no inhibition [Robson & Schild, 1937]. A vaso-constriction of the same extent produced under the same conditions by adrenaline causes inhibition of the muscle (see Fig. 5).

The balance of evidence thus suggests that vasopressin exerts a direct inhibitory action on the uterine muscle.

Summary

1. The effect of drugs on the blood flow through the uterus has been investigated. The blood flow was recorded by (1) an oncometer method, and (2) a constant rate perfusion method. The contractions of the uterus were simultaneously registered.

2. Oxytocin causes a marked muscular contraction but little or no effect on the blood flow.

3. Vasopressin causes a vaso-constriction whether accompanied by muscular relaxation or not.

4. Adrenaline causes vaso-constriction independently of whether it contracts or relaxes the uterus.

5. Cyanide and saline produce a fall in the perfusion pressure and an inhibition of the muscular contractions.

6. The results suggest that alterations in the blood flow through the uterus and in the rhythmic contractions and tone of the muscle may occur independently.

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Mr N. E. Condon gave us much valuable help in devising the oncometer and perfusion systems.

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HEPARIN AND THE FORMATION OF WHITE THROMBI

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The results of a previous investigation of the action of heparin in preventing thrombosis of blood vessels in dogs [Murray *et al.* 1936] led us to make a further study of this problem. In this paper we wish to discuss the effects of the anticoagulant upon the formation of white thrombi, which takes place with great regularity when blood is made to pass through glass, collodion or cellophane tubes. The only previous investigation of this aspect of the problem is that of Shionoya [1927]. Rowntree & Shionoya [1927], using rabbits as experimental animals, studied the accumulation of platelets when the blood flowed through a shunt made of glass and collodion tubing. Shionoya subsequently reported that heparin had no influence upon the rate of formation of white thrombi under the conditions of his experiments. We have made somewhat similar studies and find that in dogs, cats and monkeys, a definite effect of heparin can be demonstrated. Much more difficulty is encountered when rabbits are used but here also an effect can be established. The results of these experiments show that heparin exerts an effect upon the accumulation of platelets and, therefore, support the conclusions of Murray *et al.* who found that the frequency of the appearance of mixed thrombi, after mechanical or chemical injury to veins, was appreciably decreased when a solution of purified heparin was administered for adequate periods before and after the injury had been produced.

Methods, experimental results and comments

The experiments were conducted under nembutal anæsthesia. 35 mg. of nembutal per kg. were administered intravenously to the animal approximately 30 min. before the start of the experiment. Further

amounts of nembutal were given as required during the course of the experiment.

Observations upon dogs. The results collected in Table I illustrate the very definite effect which a dose of 450 units of heparin per kg. exerted upon the formation of white thrombi in dogs under nembutal anæsthesia. The shunt was composed of two glass cannulæ connected by a cellophane

TABLE I. The effect of heparin on the formation of white thrombi in dogs

Exp. no.	Control		Heparin—450 units/kg.	
	Time	Observations	Time	Observations
1*	(*a*) 2 hr. 45 min.	Not occluded—white thrombi	—	—
	(*b*) 2 hr. 30 min.	,, ,,	—	—
2	(*a*) 2 hr. 10 min.	,, ,,	—	—
	(*b*) 25 min.	Occluded—white thrombi	—	—
	(*c*) 35 min.	,, ,,	—	—
	(*d*) 1 hr. 30 min.	,, ,,	—	—
3	(*a*) 2 hr.	Not occluded—white thrombi	(*c*) 2 hr.	Patent—no thrombus
	(*b*) 50 min.	Occluded—white thrombi	(*d*) 2 hr.	,, ,,
4	(*a*) 2 hr.	Not occluded—white thrombi	(*c*) 2 hr.	,, ,,
	(*b*) 2 hr.	,, ,,	(*d*) 2 hr.	,, ,,
5	(*a*) 2 hr.	,, ,,	(*c*) 2 hr.	,, ,,
	(*b*) 2 hr.	,, ,,	(*d*) 2 hr.	,, ,,
6	—	—	(*a*) 2 hr.	,, ,,
	—	—	(*b*) 2 hr.	,, ,,
	—	—	(*c*) 2 hr.	,, ,,
	—	—	(*d*) 2 hr.	,, ,,
7	(*a*) 3 hr.	Not occluded—white thrombi	(*c*) 3 hr.	,, ,,
	(*b*) 3 hr.	,, ,,	(*d*) 3 hr.	,, ,,
8	(*a*) 3 hr. 8 min.	,, ,,	(*c*) 3 hr.	,, ,,
	(*b*) 3 hr.	,, ,,	(*d*) 3 hr.	Not occluded—some platelets
9	(*a*) 1 hr. 26 min.	Occluded—white thrombi	(*c*) 3 hr.	Patent—no thrombus
	(*b*) 37 min.	,, ,,	(*d*) 3 hr.	Not occluded—some platelets
10	(*a*) 1 hr. 54 min.	Not occluded—white thrombi	(*c*) 3 hr. 7 min.	Patent—no thrombus
	(*b*) 1 hr. 44 min.	Occluded—white thrombi	(*d*) 3 hr. 8 min.	,, ,,

* (*a*) indicates the right carotid artery and right jugular vein shunt; (*b*) the left carotid; (*c*) the right femoral artery and vein shunt; and (*d*) the left femoral.

tube 3 cm. in length and of 4 mm. bore. The overall length of the shunt was 17·5 cm.; the glass tubing had a bore of 2·5 mm. The diagram (Fig. 1) illustrates the method of installation of four of these shunts. In the twenty observations made without heparin white thrombi were observed to form and were found upon the termination of the experiment in every case. In eighteen observations in which heparin was administered the tubes were perfectly clear in sixteen cases. In two, small accumulations of platelets were observed. While these did not possess the structure of a thrombus they were, of course, the nucleus from which a thrombus might form. In every experiment a thorough search of the glass tubing

and of the cellophane tube was made. The cellophane tube was examined microscopically, usually after stained sections had been prepared. The results of this series of experiments leave no doubt that, under the conditions which we have described, heparin is effective in preventing the formation of white thrombi in anæsthetized dogs.

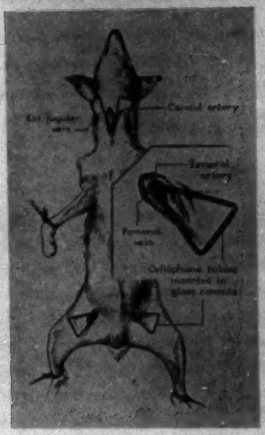

Fig. 1.

Fig. 2.

Fig. 1. Method of installation of shunts made of glass and cellophane tubing.

Fig. 2. Glass cell used for studying mechanism of formation of white thrombi. The rubber tubing connects the cell with the venous cannula. Note scratch on inner surface of cell.

Observations upon cats. The effect of heparin upon the formation of white thrombi in glass and cellophane shunts was also studied in cats. Ten observations were made without heparin and ten after injection of the anticoagulant. White thrombi were observed in every case in which no heparin was administered. The tubes became plugged after varying intervals of from 11 to 44 min. In the heparin series remarkably uniform findings were secured. Large doses of heparin were used. The loops were left *in situ* for 1 hr. and formation of thrombi was not observed in any experiment. These results are summarized in Table II.

Observations upon monkeys (Macacus rhesus). A series of observations were made upon monkeys using both the glass and cellophane loops, and

TABLE II. The effect of heparin on the formation of white thrombi in cats

	Control		Heparin—2250 units/kg.	
Exp. no.	Time taken for occlusion min.	Observations	Time loops left *in situ* hr.	Observations
1	(*a*) 11	White thrombi	(*c*) 1	Patent—no thrombi
2	(*a*) 11	,,	(*c*) 1	,, ,,
	(*b*) 17	,,	(*d*) 1	,, ,,
3	(*a*) 12	,,	(*c*) 1	,, ,,
	(*b*) 10	,,	(*d*) 1	,, ,,
4	(*a*) 12	,,	(*c*) 1	,, ,,
	(*b*) 14	,,	(*d*) 1	,, ,,
5	(*a*) 44	,,	(*c*) 1	,, ,,
6	(*a*) 20	,,	(*c*) 1	,, ,,
	(*b*) 13	,,	(*d*) 1	,, ,,

TABLE III. The effect of heparin on the formation of white thrombi in monkeys

	Control		Heparin*	
Exp. no.	Time	Observations	Time	Observations
1	(*a*) 17 min.	Occluded—white thrombi	(*c*) 4 hr.	Not occluded—white thrombi
	(*b*) 14 min.	,, ,,	(*d*) 4 hr.	,, ,,
2	(*a*) 2 hr.	Not occluded—white thrombi	(*c*) 2 hr.	Patent—no thrombi
	(*b*) 6 min.	Occluded—white thrombi	(*d*) 2 hr.	,, ,,
3	(*a*) 34 min.	,, ,,	—	—
4	—	—	(*a*) 5½ hr.	Not occluded—white thrombus started to form in 1 hr. 20 min.
5	(*a*) 11 min.	Occluded—white thrombi	(*c*) 1 hr.	Not occluded—white thrombi
6	(*a*) 9 min.	,, ,,	(*c*) 1 hr.	Patent—no thrombi
7	(*a*) 13 min.	,, ,,	(*c*) 1 hr.	,, ,,

* Exp. Nos. 1 and 2, dose = 450 units/kg.; Nos. 4–7 inclusive, dose = 2250 units/kg.

a cell composed entirely of glass. The results of the experiments in which the loops were used are summarized in Table III. Without heparin, obstruction by white thrombi took place in from 6 to 34 min., with the exception of one case in which the tube was not plugged at the end of 2 hr. White thrombi were found in all instances. With heparin, the tubes did not become obstructed. In three of the eight cases no white thrombi were found. In the other five either very small accumulations of platelets or definite small thrombi were noticed. The results indicate that heparin was exerting an effect in the prevention of the formation of white thrombi in this species.

The observations, using a glass cell, were initiated because we wished to make a motion picture in colour of the formation of white thrombi. The cell is shown in Fig. 2. The cannulæ were made of pyrex glass and were approximately 3 in. in length. The cell presented to the blood a

surface of about 4 sq. cm. Since this was much greater than in the loop previously used, one expected that thrombi would form much more easily. The process can be watched with the naked eye or through a microscope. White thrombi formed in all cases in which heparin was not used and grew to such a size that occlusion of the glass shunt was produced in all except one instance. With heparin none of the tubes became occluded and no thrombi were observed in two of the five cases. It sometimes appears that platelets accumulate on a very rough bit of the surface and that heparin is not effective in preventing this. It is possible that when the platelets are ruptured the local concentration of some substance necessary to cause them to adhere may, in some cases, be greater than that which can be neutralized by heparin in the concentration in which it is available. These results are summarized in Table IV.

TABLE IV. The effect of heparin on the formation of white thrombi in monkeys

Exp. no.	Control		Heparin*	
	Time	Observations	Time	Observations
1	(*a*) 35 min.	Occluded—white thrombi	(*c*) 4 hr. 22 min.	Not occluded—white thrombi formed within 5 min.
2	(*a*) 39 min.	,, ,,	—	—
	(*c*) 9 min.	,, ,,	—	—
3	(*a*) 5 hr. 12 min.	Not occluded—white thrombi	—	—
4	—	—	(*a*) 5½ hr.	Not occluded—white thrombi formed within 39 min.
5	(*a*) 26 min.	Occluded—white thrombi	—	—
	(*b*) 16 min.	,, ,,	—	—
	(*c*) 15 min.	,, ,,	—	—
	(*d*) 26 min.	,, ,,	—	—
6	53 min.	,, ,,	1 hr.	Patent—no thrombi
7	30 min.	,, ,,	1 hr.	Patent when excised. One small point of white thrombus formed five times during first 20 min. Nothing observed thereafter
8	35 min.	,, ,,	1 hr.	Patent—no thrombi

* Exp. No. 1, initial dose = 450 units/kg.; second dose in 2½ hr. = 1200 units; Nos. 4, 6, 7 and 8, dose = 2250 units/kg.

Observations upon rabbits. The observations made upon rabbits were most interesting. It soon became apparent that heparin was not nearly as effective under the conditions of our experiment when rabbits were used as in the case of dogs, cats or monkeys. Occlusion of the loops, either those of cellophane and glass, or of loops composed entirely of glass, was, however, never observed in experiments in which heparin was used. In our experience plugging of the cannulæ is always produced, in large part, if not completely, by the formation of white thrombi. A red

TABLE V. The effect of heparin on the formation of white thrombi in rabbits

Control

Exp. no.	Time	Observations
1	42 min.	Occluded—white thrombi started to form in 10 min.
2	25 min.	Occluded
3	18 min.	"
4	39 min.	"
5	7 min.	"
9	(a) 4 hr.	Not occluded—white thrombi started to form in 7 min.
	(b) 3 hr. 8 min.	Occluded—white thrombi started to form in 6 min.
10	(a) 26 min.	" " 11 min.
	(b) 2 hr. 26 min.	" " 36 min.
11	(a) 1 hr.	Not occluded—white thrombi formed in 8 min.
	(b) 34 min.	Occluded—white thrombi formed in 3 min.
12	(a) 1 hr.	Not occluded—white thrombi formed in 8 min.
	(b) 12 min.	Occluded—white thrombi formed in 5 min.
13	(a) 5 hr.	Not occluded—white thrombi formed in 55 min.
	(b) 5 hr.	" " 32 min.
14	(a) 24 min.	Occluded—white thrombi formed in 9 min.
	(b) —	Not done
15	(a) 43 min.	Occluded—white thrombi formed in 18 min.
16	(a) 9 min.	" " 4 min.
17	(a) 10 min.	" " 1 min.
18	(a) 11 min.	" " 1 min.
19	(a) 10 min.	" " 2 min.
20	(a) 5 min.	" " 1 min.

Exps. 1–10. Cellophane and glass loops.
Exps. 11–20. All glass, no cellophane in loops.

Heparin*

Exp. no.	Time	Observations
1	2 hr.	Not occluded—white thrombus formed
2	2 hr.	" "
3	2½ hr.	" " in 26 min.
4	2½ hr.	Patent—no thrombi
5	1 hr. 50 min.	Not occluded—white thrombi formed in 15 min.
6	(a) 2 hr. 20 min.	" " 30 min.
	(b) 2 hr. 18 min.	" " 30 min.
7	(a) 2 hr.	" " 30 min.
	(b) 2 hr.	" " 30 min.
8	(a) 2 hr.	" " 30 min.
	(b) 2 hr.	" " 30 min.
11	(c) 1 hr.	" " 15 min.
	(d) 1 hr.	" " 16 min.
12	(c) 1 hr.	" " 14 min.
	(d) 1 min.	" " 14 min.
14	(c) 1 hr.	Patent—no thrombi
	(d) 1 hr.	Not occluded—white thrombi formed in 4 min.
15	(c) 1 hr.	" " 19 min.
16	(c) 1 hr.	" " 12 min.
17	(c) 1 hr.	" " 21 min.
18	(c) 1 hr.	" " 9 min.
19	(c) 1 hr.	" " 11 min.
20	(b) 1 hr.	" " 11 min.

Exps. 1–8. Cellophane and glass loops.
Exps. 11–20. All glass, no cellophane in loops.

Exps. Nos. 1, 2, 3, 6 and 7, dose = 450 units/kg.; Nos. 4, 5 and 8, dose = 900 units/kg.; Nos. 11 and 12, dose = 2250 units/kg.; Nos. 14–20, dose = 4500 units/kg.

thrombus or clot subsequently forms in the stagnant or very slowly moving stream (Figs. 3 and 4). The white thrombi may form in any part of the tube but frequently they break away and, if large enough, plug the tip of the venous cannula. In the experiments on rabbits in which heparin was used clumps of platelets were observed to form but these never reached a size sufficient to produce plugging of the venous cannula. In only two cases in the rather large series of observations (Table V) were the tubes found completely free of white thrombi. In both cases heparin had been used. The main point of evidence, however, that heparin influenced the formation of thrombi in the experiments on rabbits, is that in no case was a thrombus formed, in a heparin experiment, which was sufficiently large to cause plugging of the tip of the venous cannula. The rabbits weighed from 1500 to 2000 g. and the diameter of the cannula used was smaller than in the other species. While we believe that an effect of heparin is apparent in these experiments, it would also appear that the conditions which encourage the accumulation of platelets are operating to such an extent that it is difficult to demonstrate its influence.

Observations upon the mechanism of formation of white thrombi. When an area in the glass cell, on which white thrombi always form in the absence of heparin, is watched through a microscope, the deposition of platelets can be readily studied. There would appear to be no doubt that the white thrombus is built up primarily of platelets as Bizzozero [1882] originally showed. If a fresh white thrombus is pulled out of the cell by means of a fine wire and quickly dissected in saline on a slide, the individual platelets can be seen (Fig. 5). Such a fresh thrombus must be obtained within a few minutes of its formation, otherwise the platelets become so tightly packed together that they lose their normal outlines. An early thrombus like this has a homogeneous granular character and stains uniformly a faint pink with hæmatoxylin and eosin staining. A thrombus that has been allowed to remain *in situ* for approximately an hour or more undergoes another change. In addition to the fusing together of the platelets one finds, in confirmation of previous workers, that they undergo a hyalinization. Staining of such thrombi indicates that a part of the platelet mass absorbs the acidophilic stains better than other portions. This results in the wavy appearance of the thrombus. More faintly staining portions of the platelet mass are frequently in close relationship to enmeshed white cells (Fig. 6). If one wishes, however, to observe the earliest alterations in blood platelets, a study of blood films under dark field illumination must be made [Ferguson, 1934]. Some of

Fig. 3.

Fig. 4.

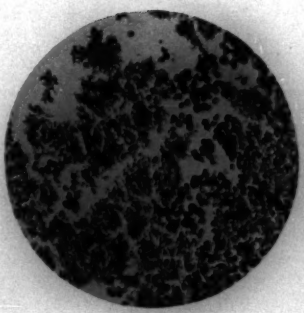

Fig. 5.

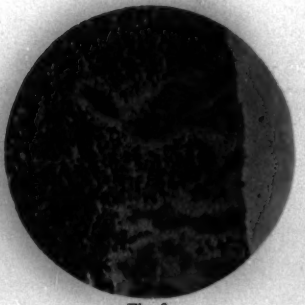

Fig. 6.

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Fig. 7.

the early changes seen in a film of human blood are shown in Fig. 7. These clumps of two or more platelets seen in the blood film (Fig. 7, No. 11) are presumably similar to those which form the nuclei of thrombi.

While a detailed study has not as yet been made of the mechanism of formation of thrombi under the conditions of our experiments, a great deal of evidence has already accumulated which confirms the conclusions of most pathologists that the thrombus grows distally or down-stream.

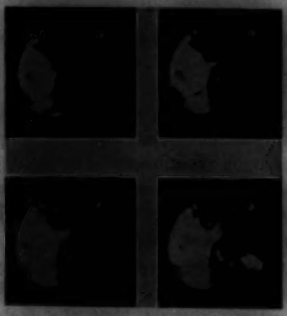

Fig. 8.

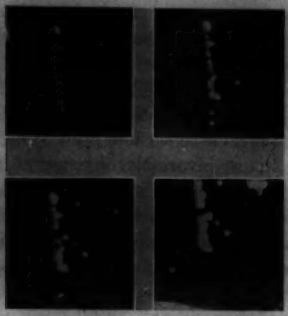

Fig. 9.

Fig. 8. Photographs of white thrombi forming in a glass cell. The blood flow was from left to right. No scratch was made on the glass.

Fig. 9. Photographs of white thrombi forming on a scratch made on the inner surface of the glass. Blood flow was from left to right.

This growth is illustrated in Fig. 8 and is even more conclusively shown in Fig. 9. These pictures were taken from a motion picture film. In the first series of pictures the inner surface of the cell had not been scratched, but in the second series the thrombi have formed upon a scratch made upon the upper surface of the glass cell (Fig. 2). There is almost no deposition of platelets up-stream from the scratch; the thrombus grows down-stream by deposition of more platelets upon those already present. While there is a very definite deposition of platelets in the periphery of this glass cell where the blood stream is slowed, this process takes place quite as rapidly on the scratch, which is in the axial stream. The flow is rapid since the glass tubing which connects the carotid artery and the jugular vein to the cell is of approximately the same calibre as the carotid artery.

Effect of heparin on the circulation. Previous investigators have studied the effect of heparin upon blood pressure and heart rate and various other indices of circulatory function. The dose of heparin which we have used in these experiments produces, at most, only a transient effect upon the heart rate and blood pressure of experimental animals. In a recent experiment on a monkey in which the changes in blood pressure and heart rate were carefully noted no significant change was observed. There was no change in the electrocardiogram. The results of these observations suggest that the effect of heparin upon formation of white thrombi is not related to an action upon the circulation.

Discussion

The results of the investigations which we have reported demonstrate the effect of heparin in preventing the formation of white thrombi, which is almost invariably observed in the control experiments. The findings are very definite in dogs, cats and monkeys but more difficulty is encountered in demonstrating this effect of heparin in rabbits. Among the many possible explanations it may be suggested that the platelets of rabbit blood are more easily destroyed under our experimental conditions than those of the other species or that they liberate a more powerful "adhesive agent". While our observations suggest that the factor responsible for the clumping of the platelets is not fibrin formed from plasma fibrinogen, direct proof of the presence of an "adhesive agent" in platelets is needed to exclude the plasma fibrin theory and also others based on the physical properties of the "third blood cell".

The structure of white thrombi has interested students since the work of Virchow [1863] who believed that the whitish mass, which is frequently observed in blood vessels under certain conditions, was formed by white blood cells, disintegrated fibrin and red blood corpuscles which had lost their hæmoglobin. We are indebted, however, to Mantegazza [1869] for the demonstration of the fact that the white thrombus does not arise by a gradual change from a red thrombus but originates in a form very similar to that in which it finally appears. Mantegazza thought the thrombus was formed of white blood corpuscles and fibrin and many of the subsequent workers had the same conception of the mode of origin. A very great advance was made in this field in 1882 by Bizzozero who watched the formation of a white thrombus under the microscope. He found that when he caused slight damage to the inner surface of an arterial wall in small mammals, a thrombus was soon produced. The whitish mass was formed almost entirely by blood platelets. There were

only a few white blood cells. It would appear that relatively little information has been added to that which was available in 1882. The findings of Bizzozero with regard to the origin of a thrombus have been supported by those of Eberth & Schimmelbusch [1886], Zurhelle [1910] and Welch [1920]. Our own observations are quite in accord with his conclusions.

Direct evidence that white thrombi grow under the conditions of our experiments by deposition of platelets on the distal or down-stream side of the primary mass is readily obtained by observing the process in the glass cell. The pictures in Fig. 8 illustrate this fact. The proximal or up-stream side of the mass of platelets changes very little. Definite growth can be noted on the right or distal side. This phenomenon is shown even more clearly when growth of the thrombi on the scratch is watched. The pictures in Fig. 9 illustrate the changes which are observed. Observations made upon the *formation* of thrombi on the scratch and also in the narrow parts of the cannula would appear to show that this process may take place without marked slowing of the blood stream. On the other hand, it is certainly true that one of the causes of thrombus formation is slowing of the stream. This, of course, has been demonstrated frequently in previous investigations and can readily be deduced from our own observations. We would like to stress the fact, however, that if there is a rough surface, such as a scratch on the glass tubing, thrombi form quickly in spite of the fact that the blood stream is moving very rapidly. It may be argued with justice that growth of these thrombi is due, in part, to a deposition of platelets in the eddy formed in the stream by the first deposit of cells. Whether or not the scratch produces invisible eddy currents (certainly none can be seen until clumps of platelets are visible) and in this or some other manner produces a local slowing of the stream which is responsible for the original deposition of platelets, cannot be decided at present.

Summary and conclusions

1. When a shunt composed of glass or cellophane tubing is inserted between an artery and a vein in anæsthetized dogs, cats, monkeys or rabbits, large white thrombi rapidly form and, in many cases, completely obstruct the flow of blood.

2. The administration of a large dose of purified heparin usually prevents or delays the formation of these thrombi in dogs, cats or monkeys. In rabbits growth of the thrombi is apparently inhibited, but

very small clumps of platelets may form even when large doses of heparin are used.

3. The use of a transilluminated glass cell has made it possible to record the formation and growth of white thrombi on photographic film.

4. The results of this study show, in confirmation of those of previous investigators, that the thrombi usually grow down rather than up the blood stream from the original focus.

We are indebted to our colleagues Dr Arthur Charles and Dr James Craigie for a great deal of help in these studies. The purified heparin was made by Dr Charles in the Connaught Laboratories. Dr Craigie has been responsible for most of the photographic work.

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DESCRIPTION OF PLATES I AND II

PLATE I

Fig. 3. White thrombus which lodged in venous cannula in one of the experiments on dogs. The red thrombus or clot formed later.

Fig. 4. Cross-section of white thrombus seen in Fig. 3. The darker areas are masses of red blood cells. There is some fibrin and a few white blood cells but most of the section is made up of platelets. (H. and E. stain ×50.)

Fig. 5. Photomicrograph of fresh white thrombus dissected in saline. The larger cells are erythrocytes. Some individual platelets are shown but even these bear little resemblance to those seen in a fresh film of blood. (H. and E. stain ×325.)

Fig. 6. Section of a compact white thrombus. The largest cells are leucocytes. The more homogeneous lighter coloured areas are composed almost entirely of platelets. (H. and E. stain ×183.)

PLATE II

Fig. 7. Photomicrographs of human platelets observed under an oil immersion lens with dark field illumination. (Magnification × 1475.)

1, 2, 3. Platelets as seen early in an unstained saline preparation. An outer thin portion with an irregularly indented margin surrounds the central thicker portion. The thicker portion has refractile granules in it.

4, 5, 6, 7. As the platelet ages in these preparations there is an apparent deepening of the indentations until finally the platelet consists of a central refractile area surrounded by hair-like processes.

8, 9. A later stage is seen here, in which the beginning of the vacuolar type of degeneration is occurring. No. 9 is a photograph taken 5 min. after No. 8 of the same platelet.

10. This shows fine vacuoles appearing along the hair-like processes.

11. At a more advanced stage of degeneration the platelets appear as a mass containing numerous vacuoles or globules. These may be separated at times more than is shown here.

12. A white blood cell photographed under the same conditions of lighting and with the same magnification used in making the pictures of the platelets.

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A NOTE ON THE EFFECTS OF VITAMIN A DEFICIENCY IN PREGNANT RATS

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In his paper entitled "Fœtal death, prolonged gestation, and difficult parturition in the rat as a result of vitamin A deficiency", Mason [1935] gives an excellent review of previous work on the subject, and an account of his own comprehensive investigation. Tansley [1936] also notes the frequent occurrence of long pregnancy and difficult labour in rats deficient in vitamin A. Mason states that during early pregnancy localized areas of infection, leucocytic infiltration and cellular necrosis occur at the junction of the ectoplacenta and maternal decidua, leading to fœtal death and reabsorption. The process is easily distinguishable from that following deficiency in vitamin E. Failing reabsorption, a second phenomenon observed is delayed fœtal development, followed by death of the fœtuses later in pregnancy. The third possibility, classed, with reservations, as a sequel to the second, is the delivery of dead fœtuses 2 or 3 days after the normal time for parturition, frequently attended by excessive hæmorrhage or the discharge of foul-smelling fluid, and lasting up to 2 days. This is regarded as a less severe manifestation of the same infective and degenerative processes previously described; but difficult labour is sometimes attended by the delivery of live fœtuses, and dead ones found post-mortem in the uterus may occasionally exceed the weight of normal full-term fœtuses. Mason does not attempt to explain fully, on his findings, the causes of the difficult labour, and both he and Tansley note, among other things, the flaccidity of the uterus.

For these and other reasons the present author thought that uterine inertia, occurring as a primary muscular deficiency, or secondarily as a result of nerve degeneration or abnormal hormonal influences, might account for the findings, and that the physiological behaviour and

reactions of the muscle at parturition would repay study. Accordingly, a preliminary investigation, the results of which are here recorded, was carried out to find the optimal conditions for obtaining difficult parturition. Unfortunately neither Mason nor Tansley gives the proportion of uncompleted pregnancies (due to reabsorption, etc.) to be expected in different degrees of deficiency.

The present investigation shows that, given routine treatment by groups, the occurrence of difficult parturition is fortuitous and relatively infrequent compared with uncompleted pregnancy. This does not exclude the possibility that skilled dietary treatment of each individual rat might bring about the desired result more often, but it is disappointing in view of the main object which was to produce large numbers of difficult labours, and the expectation that these would result from a certain level of deficiency.

Methods and results

The rats from which the colony was bred were kindly supplied by the Lister Institute, and the breeding stock was maintained on the Lister Institute regime. This included a liberal and varied diet, scrupulous cleanliness, and a room temperature of not less than 70° F. The vitamin A-deficient diet was the same as that used by Tansley [1936].

Weaning took place 3 weeks after birth, and the rats were mated as soon as their vaginas opened. Pregnancy occurred at an average age of 12 weeks (normal rats) or 13 weeks (A-deficient rats). Vaginal smears were made daily, and the day on which spermatozoa were found was taken as zero in computing the duration of pregnancy.

Various degrees of deficiency in vitamin A were produced by allowing batches of rats a normal diet for different periods after weaning, and then placing them on the deficient diet. To control possible variations in the breeding stock, litters were given the longer or shorter normal diets indiscriminately at all periods of the investigation.

Full records of all pregnancies were made, but with one possible exception, no observation was made to conflict with or add to Mason's excellent account of the effects of deprivation of vitamin A on gestation and parturition. Only the numerical results are of interest, and these are presented in an abbreviated form in Table I, which has been made, as far as possible, self-explanatory. A similar table (not reproduced) was constructed in which the first column gave the length of time on deficient diet preceding pregnancy, instead of the period on normal diet as in

TABLE I

Description of rats			Pregnancy				Litters		
Days old when put on A-deficient diet	Further treatment during pregnancy	Total number	Normal	Prolonged, otherwise normal	Ending in difficult labour	Uncompleted	Average no. in litters (excluding cols. 6 and 7)	P.c. dead foetuses in litters	Average weight of foetuses g.
1	2	3	4	5	6	7	8	9	10
35–45	Nil	22	4	3	3	12	8·0	25	4·5
	*E	12	3	2	2	5		No details	
	*A	8	8	0	0	0	9·1	5	5·0
46–50	Nil	16	8	4	1	3	9·4	33	4·5
	*E	13	6	0	3	4	9·8	14	4·8
	*A	4	3	1	0	0	10·0	15	5·0
51–65	Nil	5	5	0	0	0	8·6	9	4·8
	*E	7	4	1	0	2	7·8	10	5·3
	*A	9	7	2	0	0	9·1	5	4·8
Normal diet throughout exp.		26	22	1	0	3	8·7	3	5·2

In three further rats of the 35–45-day group, carotene administration was started after one-third of pregnancy had elapsed. In only one was pregnancy completed, with a normal litter of five alive and one dead, average weight 4·9 g.

For details of twenty rats kept on A-deficient diet from date of weaning, see text.

Notes: (1) *E indicates administration of 5 g. wheat germ daily per rat. *A indicates administration of 5 γ carotene daily per rat.

(2) Of the twenty-two rats in col. 4 of the normal group, nine littered on the 21st and thirteen on the 22nd day of pregnancy. Almost without exception, the deficient rats recorded in the same column littered on the 22nd day. Parturitions occurring on or after the 23rd day are recorded in col. 5.

(3) All the pregnancies recorded, except eight, were first pregnancies.

Table I. There was a slightly greater proportion of normal pregnancies after the short periods of deficiency than after the long, but this was sufficiently accounted for by the fact that the reason for the shorter period was usually not an earlier mating, but a longer time on normal diet. Absolutely no relation was found between arbitrary numerical combinations of the time on normal diet and the time on deficient diet which could not be explained by the relation shown in Table I.

It should be mentioned at this point that the actual degree of deficiency in vitamin A exhibited by the rats was of secondary interest in these preliminary experiments: it was desired merely to cover empirically the range of deficiency producing abnormalities of pregnancy and parturition in first pregnancies. The determining factor was the length of time the rats had been on normal diet in the first place, but the author does not feel competent to say whether the animals were in a steady state of deficiency at the time of pregnancy. For those who use weight as a criterion, it may be of interest that the average weights of the rats at the time when deficient diet was instituted were 55 g. in the first group, 75 g. in the second group, and 75 g. also in the third group. This last weight should have been about 100 g., but by chance, several poor litters were included. It will be observed from the table that this subnormal weight did not appear to affect the performance of the group during pregnancy, thus emphasizing the special effect of vitamin A. The rats of all three deficient groups weighed 135–145 g. at an age of 3 months, but the weight

curves were flattening out at this time, and very few of the rats were approaching 200 g. weight at an age of 4 months. Xerophthalmia was not observed, but respiratory infection was at times difficult to control.

Wheat germ was added during pregnancy to the diet of some of the groups (5 g. per day per rat) to exclude deficiency of vitamin E as a cause of the uncompleted pregnancies which were being found. It will be seen that it made no appreciable difference, while carotene (5γ per rat per day during pregnancy) was sufficient alone to remove all abnormalities.

Of the twenty-eight uncompleted pregnancies in the deficient animals, eight were definitely due to reabsorption or abortion, twelve showed no trace of placental sites at post-mortem on about the 20th day after copulation, and had previously exhibited no signs of pregnancy, while eight were not examined. With regard to the twelve presumed not to have been pregnant, it should be pointed out that the bucks were not sterile, were kept on a normal diet, and were placed with the females only at night. Also the incidence of uncompleted pregnancy was nil in the carotene-treated rats. Mason, treating the point incidentally, states that infertile matings are no more frequent in A-deficient than normal animals, but the findings here recorded, though negative in character, agree with previous work by Evans [1928]. The three cases of uncompleted pregnancy in the normal group of rats were not subjected to post-mortem examination, though the weight curves suggest that they had been pregnant.

Of the twelve cases of difficult labour observed, three were allowed to recover, four showed no apparent sepsis, apart from possible secondary infection of the necrotic fœtal material in the uterus, but five exhibited definite infection of the uterus itself or the surrounding tissues. This, together with the relatively small proportion of difficult labours observed, renders doubtful the possibility of a clean investigation of the uterine muscle. On the other hand, a few labours which appeared difficult, but which were completed within an arbitrary time limit of 2 hr., are included in col. 5 of Table I.

The three rats exhibiting difficult labour which are not mentioned in Table I were members of a final group of twenty animals which were placed on a deficient diet + wheat germ immediately on weaning. They were mated as usual, and smeared for several weeks, but no spermatozoa were found; cornified cells appeared in the vaginal smear in large numbers, and the animals showed lack of growth and other evidences of A deficiency. Vaginal examinations were abandoned, and the rats examined twice weekly. Some of them died from respiratory infection

and inanition, but eventually four were found in an advanced state of pregnancy. One of these died before reaching full term, and the remaining three had difficult labours. It is impossible to say how many uncompleted pregnancies had occurred, but the main defect was failure to copulate [cf. Parkes & Drummond, 1926].

Summary

The incidence of abnormal parturition and other disturbances of pregnancy has been investigated in 120 rats in various states of deprivation of vitamin A. Uncompleted pregnancy is much more frequent than difficult labour, and this is often complicated by infection of the uterus. Sufficient material for an exploratory study of the uterine muscle during difficult parturition would not be easy to obtain. Various observations on pregnancy in rats deficient in vitamin A are briefly recorded, and are for the most part in agreement with the findings of Mason [1935].

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CHOLINE ESTERASE IN VOLUNTARY MUSCLE

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The theory of chemical transmission of motor nerve impulses to voluntary muscle—suggested by Dale and his co-workers—encountered as a chief difficulty, that the chemical process involved must occur with a flashlike suddenness. The acetylcholine (ACh.) liberated at the nerve endings must be removed in the very brief limits of the refractory period [Brown *et al.* 1936]. The necessity for so great a rapidity is a main difference between the transmission of nerve impulses in quickly reacting cells like the fibres of striated muscle and the transmission from the autonomic system to the effector cells.

The problem has been attacked by measuring the rate of ACh. hydrolysis by choline esterase in voluntary muscle [Marnay & Nachmansohn, 1937*a*]. These experiments indicated that the concentration of the enzyme in muscle tissue is not high enough to remove during the refractory period such an amount of ACh. as may be liberated by a single motor nerve impulse. It was calculated that the time necessary for the hydrolysis of 50 p.c. of such an amount was many thousand times longer than the refractory period, at a concentration of the enzyme as found in muscle tissue. The conclusion was drawn that, if the disappearance of ACh. at the end-plates during the refractory period was due to the activity of the esterase, a concentration of the enzyme at the nerve endings is necessary which is many thousand times as high as that found in muscle tissue.

In this paper it will be shown that a high concentration at the end-plates, such as postulated in the previous paper, does in fact exist. The rapidity of the hydrolysis of ACh. is, therefore, as high as is required by the hypothesis that this substance is involved in the transmission of motor nerve impulses to voluntary muscle.

Methods

In the previous experiments chopped muscle pieces or slices of tissue were put into Ringer solution containing acetylcholine. After a suitable time an aliquot amount of the suspension fluid was withdrawn and used for the manometric determination of ACh. Some of the experiments described in this paper were carried out by this method. In most of the experiments, however, the tissue was ground with quartz. The rate of hydrolysis can thus be determined directly by measuring the output of CO_2 liberated from the bicarbonate of the solution by the acetic acid formed. Under these conditions the output of CO_2 corresponds to the amount of ACh. hydrolysed, whereas in intact cells other metabolic processes interfere. The procedure was as follows: a weighed amount of muscle was thoroughly ground in 1 c.c. of Ringer solution with a small quantity of quartz in a small porcelain mortar. The suspension was transferred to a Warburg vessel, the mortar being washed twice with 1 c.c. of Ringer solution. The ACh. solution was run into the side bulb. After equilibrium had been attained in the thermostat, acetylcholine was tipped in. In the suspensions from some tissues—especially nerves—there sometimes occurs a slight uptake of CO_2 continuing for a long time (up to several hours) in the absence of ACh., so that a control vessel with tissue extract without ACh. is necessary.

If a solution or suspension in the manometric vessels contains substances binding acids, as proteins or secondary phosphates or other buffers, the output of CO_2 does not correspond exactly to the CO_2 or to the acids formed: a fraction of the acid is bound by the buffer substances [Warburg, 1925]. The amount of retained CO_2, the "retention", must be determined and a factor must be added to the normal constant of the vessel to obtain the real amount of acid formed.

Suspensions of ground muscle in Ringer solution contain such retaining substances. The retention varies a little for the different tissues and the different conditions as temperature, *p*H, etc. For frog's muscle at 19° the following values for $\left(\frac{\Delta u}{\Delta p}\right)_{acid}$ have been found if 100 mg. were ground up in 3 c.c. of Ringer solution: 0·035, 0·044, 0·038, 0·022 and 0·037; mean value 0·035. The factor is proportional to the weight of muscle: for 200 mg. ground in 3 c.c. of Ringer solution, the factor rises to the mean value of 0·079 (0·080, 0·093, 0·106, 0·076, 0·053 and 0·070 are the values found).

The Ringer solutions had the same composition as described in our previous paper. All experiments were carried out in nitrogen containing

5 p.c. CO_2. The initial concentration of ACh. was always 1 mg. per c.c. The frogs used were *Rana esculenta*.

Phosphates and phosphorylated substances were determined with the hydrolytic method of Lohmann [1928]. In these experiments the muscles were cut and immediately frozen in liquid air.

Results

Choline esterase in frog's sartorius. The nerveless pelvic end of frog's sartorius has often been used for investigations of the properties of muscle tissue [Lucas, 1907; Lucas & Mines, 1907; Lapicque, 1931; Rushton, 1931]. To secure evidence for an accumulation of choline esterase at the nerve endings, the enzymic activity of the nerve-free part was compared with that of the part containing nerve endings.

The amount of acetylcholine hydrolysed per unit of weight in 1 hr. by a quarter of the sartorius containing nerve endings is about threefold the corresponding quantity for the pelvic end [Marnay & Nachmansohn, 1937*b*]. As the volume of the end-plates constitutes only a very small fraction of the whole muscle, such an increase of the total hydrolytic power indicates a very high concentration of the enzyme in this small volume.

The difference of the concentration of enzyme between the nerveless part and that containing nerve endings can be shown in an even more striking manner. The distribution of the nerve endings in frog's sartorius has been studied recently in a very complete manner by Pézard & May [1937]. These authors found that only about an eighth of the pelvic end is really nerveless. About one-fifth contains practically no nerve endings. The second fifth is very rich in nerve endings and then the frequency decreases considerably. The second fifth contains about twice as many nerve endings as the third fifth.

The comparison of the time-course of hydrolysis between 1/8 to 1/6 of the pelvic end and the second and third fifth shows a plain parallelism between richness in nerve endings and rate of hydrolysis. The second fifth, containing the greatest number of nerve endings, hydrolyses acetylcholine about 6 times as quickly as the nerveless part, whereas the third fifth hydrolyses only about 3 times as quickly (see Fig. 1). Table I summarizes the results of four experiments, each made with the corresponding parts of four muscles. These experiments were carried out with ground tissue.

The rate of hydrolysis by the sciatic nerve is about six- to sevenfold that in the muscle (nerveless part). There is practically no difference

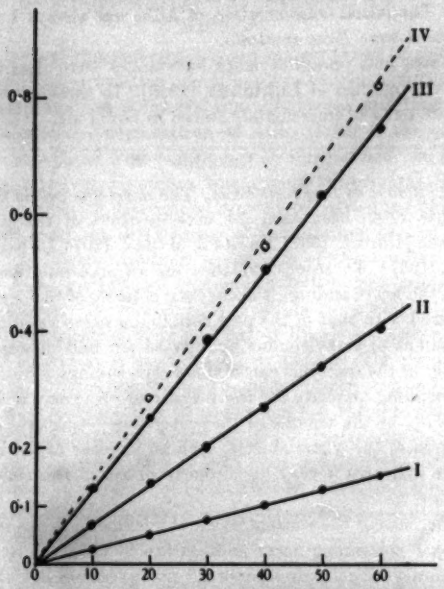

Fig. 1. Rate of hydrolysis of ACh. by different parts of frog's sartorius. Abscissa: time in min. Ordinate: mg. ACh. hydrolysed by 100 mg. of tissue.

I. Nerveless pelvic end.
II. Third fifth.
III. Second fifth.
IV. Sciatic nerve (broken line).

TABLE I. Choline esterase in frog's sartorius

	Nerveless end		Second fifth		Third fifth		Nerve (sciatic)	
No.	Muscle weight mg.	ACh. hydrolysed by 100 mg. in 60 min. mg.	Muscle weight mg.	ACh. hydrolysed by 100 mg. in 60 min. mg.	Muscle weight mg.	ACh. hydrolysed by 100 mg. in 60 min. mg.	Weight mg.	ACh. hydrolysed by 100 mg. in 60 min. mg.
1	97	0·152	114	0·745	137	0·402	54·5	0·893
2	83	0·135	111·5	0·850	132	0·432	56	0·705
3	90·5	0·131	98·5	0·890	102	0·485	66	0·807
4	102	0·123	121	0·695	134	0·298	38	0·868
5	—	—	—	—	—	—	36	0·816
Mean value		0·135		0·795		0·404		0·818

between the rate of hydrolysis by the nerve and the part rich in nerve endings. $\left(\frac{\Delta u}{\Delta p}\right)_{acid}$ for 100 mg. of nerve tissue ground in 3 c.c. of Ringer solution is the same as that for muscle tissue: 0·034 and 0·036 are the values found; mean value 0·035.

Diffusion through muscle tissue. In the previous paper it was shown that the time-course of hydrolysis of acetylcholine by intact frog's muscle of 400 mg. fresh weight does not differ considerably from chopped muscle pieces of about 30 mg. fresh weight. These experiments seemed to indicate that a diffusion of acetylcholine through muscle tissue occurs rather quickly.

The rate of hydrolysis by sartorius containing nerve endings is only a little higher than that by the nerve-free part of the muscle if small muscle pieces are used. Table II shows that the amount of acetylcholine

TABLE II. Difference of enzyme activity between ground tissue and muscle pieces

A. Nerveless end

	(*a*) Pieces of about 30 mg. fresh weight		(*b*) Ground tissue	
No.	Weight mg.	ACh. hydrolysed by 100 mg. in 120 min. mg.	Weight mg.	ACh. hydrolysed by 100 mg. in 120 min. mg.
1	189	0·238	97	0·308
2	230	0·283	83	0·279
3	263	0·312	90·5	0·269
4	198	0·116	102	0·246*
5	255	0·169	127	0·260
6	152	0·210	—	—
7	165	0·266	—	—
8	224	0·139	—	—
9	200	0·195	—	—
	Mean value	0·214		0·272

B. Second fifth

1	188	0·250	114	1·490†
2	157	0·344	111·5	1·700†
3	187	0·266	98·5	1·780†
4	—	—	121	1·390†
	Mean value	0·285		1·590

* Calculated from a value found for 60 min.
† Calculated from the values for 60 min. given in Table I.

hydrolysed by pieces rich in nerve endings (second fifth of sartorius) is only about 30 p.c. higher than by pieces of the nerve-free part (mean value 0·285 mg. ACh. hydrolysed by 100 mg. in 120 min. against 0·214

mg.). The difference appears however if the muscle pieces are ground: the amount hydrolysed by 100 mg. of nerve-free tissue rises only from 0·214 to 0·272 mg., whereas the amount hydrolysed by muscle containing nerve endings rises from 0·285 to 1·590 mg.

The difference in the rate of hydrolysis between ground muscle and muscle pieces of the nerve-free part indicates that the rate of hydrolysis is delayed by diffusion through muscle tissue. As the enzymic activity at the nerve endings is much higher than that in muscle tissue itself, the diffusion to these spots is probably not quick enough to saturate their enzyme with substrate and therefore the delay in the rate of hydrolysis brought about by diffusion is much greater in muscle containing nerve endings. If such muscles are ground, the enzyme of the nerve endings comes in close contact with the substrate and works now at the maximal rate. This may explain the big increase of the rate of hydrolysis.

The experiments with muscle pieces were carried out under exactly the same conditions as the experiments with ground muscle. The pieces were put into Warburg vessels containing 3 c.c. of Ringer solution. The ACh. concentration was the same (1 mg. per c.c.). The vessels were shaken during 2 hr. in the thermostat and then the ACh. that remained and the initial amount determined as previously described. The time of 2 hr. was chosen in order to obtain more exact results because the absolute amount hydrolysed after 1 hr. would not be great enough, as the initial amount (3 mg.) is comparatively high in view of the small quantity of muscle used.

Dog's gastrocnemius. It can also be shown with dog's gastrocnemius that the distribution of choline esterase in voluntary muscle is not even, but differs according to the distribution of the nerve endings. Small pieces of 30–50 mg. can be obtained in which no nerves can be found as can be controlled microscopically.[1] By following nerve ramifications as far as possible with a magnifying glass it is possible to obtain pieces containing nerve endings. Although the nerve endings constitute only a very small fraction of these pieces, the rate of hydrolysis is about twice as high as in the nerve-free pieces. This is shown in Table III. If the pieces are too big, as in No. 2*e*, the amount hydrolysed is only a little increased, the part played by the nerve endings becoming too small. On the other hand in one experiment (No. 3*c*) it was possible to obtain a very small piece containing nerve endings which yielded a particularly high rate of hydrolysis.

[1] We are much obliged to Dr Couteau from the Laboratoire de Biologie Expérimentale, who has carried out the microscopical examinations for these experiments.

TABLE III. Dog's gastrocnemius (ground with quartz)

No.	Part of muscle	Muscle weight mg.	ACh. hydrolysed by 100 mg. in 60 min. mg.
1 (*a*)	Nerveless	24	0·58
(*b*)	Nerveless	31	0·48
(*c*)	Containing nerve endings	20·5	1·05
2 (*a*)	Nerveless	34	0·34
(*b*)	Nerveless	42·5	0·36
(*c*)	Nerveless	45	0·36
(*d*)	Containing nerve endings	17	0·81
(*e*)	Containing nerve endings	87	0·47
3 (*a*)	Nerveless	30	0·49
(*b*)	Containing nerve endings	18	0·97
(*c*)	Containing nerve endings	6	1·35

The experiments were made with ground muscle at a temperature of 37°. The retention of CO_2 was determined with 50 mg. of muscle ground in 3 c.c. of Ringer solution. With this amount $\left(\frac{\Delta v}{\Delta p}\right)_{acid}$ is 0·044.

Phosphorylated substances in frog's sartorius. It seemed to be of interest to investigate if there are other substances which are not evenly distributed in frog's sartorius, or if this distribution is peculiar to choline esterase. The concentration of phosphates and phosphorylated substances has been determined, as these compounds have so many different functions in the glycolysis. Table IV shows that no differences

TABLE IV. Distribution of phosphorylated substances in frog's sartorius (mg. P_2O_5 per g. muscle)

No.	Weight g.	Phosphagen	0 min.	7 min.	30 min.	Adenyl-pyro-phosphate	180 min.	Total P_2O_5	Hexose-mono-phosphate	Hexose-di-phosphate
1 (*a*)	0·473	0·69	2·09	2·49	2·51	0·38	3·23	3·48	0·35	0·66
(*b*)	0·497	0·76	2·17	2·61	2·65	0·40	3·31	3·55	0·34	0·64
2 (*a*)	0·421	0·55	1·94	2·35	2·43	0·33	3·04	3·34	0·42	0·65
(*b*)	0·471	0·48	1·88	2·36	2·46	0·38	3·01	3·23	0·32	0·65
3 (*a*)	0·469	0·73	1·83	2·58	2·65	0·68	3·14	3·29	0·21	0·57
(*b*)	0·533	0·60	1·96	2·78	2·83	0·77	3·18	3·37	0·27	0·37

(*a*) Nerveless end. (*b*) Second fifth.

of concentration exist between the nerveless pelvic end and the second fifth particularly rich in nerve endings. The concentration of phosphagen is rather low and indicates that a fraction of the initial amount is hydrolysed, probably during the cutting of muscle as would be expected. The directly determined phosphate however, which is the sum of phosphagen phosphate and inorganic phosphate, is equal in the two parts (column

0 min.). A difference in the initial amounts of phosphagen is therefore not probable.

Lizard's muscle. The lizard is an animal capable of very quick movements, and its nerve endings, described by Rouget [1862] as "plaques terminales", are quite different from the nerve endings in frog's muscle. They resemble the end-plates in mammalian muscle and are even larger. In view of these special functional and structural properties it seemed to be of interest to know if there is a difference between the concentration of choline esterase in this muscle and that in other muscles.

The experiments were carried out with ground muscle tissue of the hindlimb and the tail of the green lizard (*Lacerta viridis*). The retention of CO_2 after grinding 100 mg. of these muscles in 3 c.c. of Ringer solution is the same as that of frog's muscle. $\left(\frac{\Delta w}{\Delta p}\right)_{acid}$ under these conditions was found to be 0·018, 0·037, 0·038; mean value 0·031 (for frog's muscle 0·035).

Table V gives the data obtained. The amount hydrolysed in 1 hr. by 100 mg. of muscle is indeed very high, 3–5 times that by frog's muscle and more than twice that by mammals at 37° (cat, dog and guinea-pig).

TABLE V. Lizard's muscle

No.	Part	Muscle weight mg.	ACh. hydrolysed by 100 mg. in 60 min. mg.
1	Tail and hind limb	41	1·60
		44	1·44
		57	1·35
2	Hind limb	42	2·62
		50	2·77
	Tail	47	2·74
		39	2·72
3	Hind limb	35	2·72
		44	2·83
4	Hind limb	47	3·40
		48	2·58
5	Hind limb	55	2·92

Whilst 100 mg. of frog's muscle split 0·4–0·6 mg. ACh. in 1 hr. at 18° and 100 mg. of guinea-pig's muscle 0·8–1·0 mg. at 37°, 100 mg. of lizard's muscle at 20–22° split 1·5–3·0 mg. ACh. in the same time.

Eserine completely inhibits the esterase in a concentration of $1:10^{-5}$ g. per c.c. of Ringer solution.

Discussion

The main result of these experiments is the fact that choline esterase is not uniformly distributed in muscle tissue but is concentrated at the nerve endings or in their neighbourhood. This conclusion is based chiefly upon the observation that 100 mg. of nerveless muscle tissue—frog's sartorius—split only 0·135 mg. of ACh. in 1 hr., whereas 100 mg. of the same muscle containing nerve endings split 0·4–0·8 mg. during the same time. It is improbable that the increased esterase activity is due to the presence of nerve fibres. The nerve fibre of the frog (sciatic) contains only 6 times as much esterase as the nerveless muscle tissue. Even if the volume of nerve fibres should constitute 2–3 p.c. of the total volume of the muscle (and probably the fraction is much smaller), the total amount of esterase of muscle would only be increased by 12–18 p.c. Actually the esterase activity of muscle with nerve endings is 300–600 p.c. that of the nerve-free part. It may be argued that the myelin sheath contains no enzyme at all and that therefore the concentration of the esterase in neuro-fibrils may be more than 6 times as high as that in muscle tissue, as the rate of hydrolysis was determined by grinding a part of the sciatic nerve enveloped by myelin. But even assuming 2 or 3 times the amount of esterase in the neuro-fibrils, the concentration would not be sufficient to explain the great difference between the nerve-free end and the part containing nerve endings. The improbability of such an interpretation is shown by the fact that there is no difference between the part rich in nerve endings and the sciatic nerve. If the great hydrolytic power of this part of the muscle were due to the neuro-fibrils, it would be necessary to assume that the fraction of volume taken by the neuro-fibrils in this part of the muscle and in the sciatic is approximately the same, and this is obviously impossible. It can therefore be concluded that there is a special accumulation of the enzyme between nerve and muscle, i.e. in the end-plates. This interpretation is emphasized by the striking parallelism between the hydrolytic power and the number of nerve endings.

It is impossible at present to give exact data of the fraction which the volume of end-plates constitutes in the whole organ. But let us assume that the fraction constitutes one-thousandth. An increase of the total esterase activity to 3–6 times, due to the concentration of enzyme in this fraction, would mean that at these spots the enzyme concentration must be increased 3000–6000 times. The nerve endings probably occupy less than 1/1000 of the total volume. Some estimations, although rough

and approximative, suggest that their volume is less than 1/5000. For this value a 3–6 times increased esterase activity would correspond to a concentration which is 15,000–30,000 times higher than in nerveless muscle tissue.

It is possible to calculate from the data given the approximate amount of ACh. which can be hydrolysed in the refractory period at the nerve endings of a single frog's sartorius of 300 mg. On the average 100 mg. of muscle containing nerve endings split at least about 0·4 mg. ACh. in 1 hr. 0·135 mg. is the amount hydrolysed by nerveless muscle tissue in this time. As the difference is essentially due to the esterase accumulated at the nerve endings, about 0·265 mg. ACh. can be hydrolysed there by 100 mg. muscle in 1 hr. This means that an amount of about 0·001 μg. ACh. can be hydrolysed at the nerve endings of a frog's sartorius of 300 mg. in 5 msec. (the refractory period). As about 600 nerve endings exist in a sartorius [Pézard & May, 1937] the amount which can be hydrolysed in a single motor nerve ending during the refractory period is about 0·000002μg. This amount corresponds to 8.10^9 molecules.

Two conclusions can be drawn from our experiments: first, the chemical changes at the end-plates of voluntary muscle can occur with the flashlike suddenness necessary for a chemical transmission of nerve impulses; secondly, a concentration of a specific enzyme at the nerve endings, many thousand times as high as that in the surrounding muscle tissue, must have a functional significance, and suggests—without anticipating the function—that ACh. intervenes in the transmission. The considerable difference of the concentration of choline esterase between frog's and lizard's muscle can also be interpreted in favour of its functional significance.

Summary

1. The rate of hydrolysis of acetylcholine by the nerveless pelvic end of a frog's sartorius was compared with that of the parts containing nerve endings. 100 mg. of the nerve-free ground muscle tissue split only 0·13 mg. of ACh., whereas 100 mg. of the same muscle containing nerve endings split 0·4–0·8 mg., according to the richness in nerve endings.

2. These experiments indicate the presence of a concentration of choline esterase at the end-plates which is many thousand times as high as that found in muscle tissue, enabling the muscle to split the ACh. liberated by the nerve impulses during the refractory period. The chemical changes can, therefore, occur with the rapidity necessary for the assumption of a chemical transmission of nerve impulses in such quickly reacting cells as fibres of voluntary muscle.

3. From the data given it can be calculated that about 0·001 μg. ACh. can be hydrolysed at the nerve endings of a frog's sartorius weighing 300 mg. during the refractory period. In a single motor nerve ending about 0·000002 μg. can be hydrolysed in this period, that is 8.10^9 molecules.

4. In the dog's gastrocnemius also, the choline esterase is unevenly distributed in muscle tissue.

5. The rate of hydrolysis by ground muscle is only a little higher (about 30 p.c.) than by muscle chopped into pieces of about 30 mg. The difference between the esterase activity of ground muscle and of muscle pieces increases considerably if the tissue contains nerve endings, the rate of hydrolysis being several times as high in the ground muscles. This difference is probably due to the delay occasioned by diffusion of ACh. through muscle tissue to the nerve endings, the enzyme there being unsaturated with substrate if muscle pieces are used.

6. Phosphates and phosphorylated substances have been estimated both in the nerve-free end of frog's sartorius and the part rich in nerve endings, with a view to discovering whether there are other substances showing an unequal distribution. No difference of concentration has been found between the two parts of muscle.

7. The rate of hydrolysis of ACh. by lizard's muscle is three- to five-fold that produced by frog's muscle and two- to threefold that produced by mammalian muscle at 37°.

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ON THE BEHAVIOUR OF GLYCOGEN AFTER DIETS RICH IN PROTEIN AND IN CARBOHYDRATE

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It is a well-known fact that the carbohydrate reserves that are built up in the liver from the carbohydrates of the food are very variable [Barbour *et. al.* 1927; Cori & Cori, 1928; Silvette & Britton, 1932]. As the result of hunger, of cold, of work and of certain effects of poisoning, they can be made almost to vanish. We report experiments which show that the carbohydrate reserves of the liver laid down on a protein diet behave differently from those deposited on a diet of carbohydrate.

Experimental

Animals. Where not expressly stated otherwise, male rats of our own breeding were used. In all the experiments we have examined animals of various ages but, with the exception of the hunger experiments of long duration, we have used chiefly animals of about 100 g.

Diet. The first series of experiments in each group was carried out with a natural diet composed as follows: the rats on a diet rich in carbohydrate received wheat and bread moistened with milk, those on a protein-rich diet received fresh minced meat.

The further experiments were carried out with synthetic diet. The usual diet rich in carbohydrates had the following composition: 20 p.c. casein (Glaxo), 70 p.c. starch (in some experiments 50 p.c. starch and 20 p.c. cane sugar), 10 p.c. fat (olive oil). The protein-rich diet consisted of 90 p.c. casein and 10 p.c. fat, or more frequently of 70 p.c. casein, 20 p.c. starch and 10 p.c. fat. To every 100 g. synthetic diet 3 g. salt mixture, 2 g. yeast and 25 drops of cod liver oil were added. The forms of diet are briefly denoted according to the percentage content of casein.

The animals remained in general 5–12 days on the appointed diet before they were put on experiment. Further forms of diet are stated in the special experiments. Only animals that throve well were used. The growth of the animals on a protein-rich diet was often retarded.

Temperature. The majority of the experiments were carried out in winter at a room-temperature of 16–20° C. We have repeated many experiments in the summer months (May–August inclusive) at an average room-temperature of 27° C. Qualitatively, the results were the same. Quantitatively, the experiments were far more variable. The protein animals throve with us better at a mean temperature of 18° C. than in summer.

Methods. Blood sugar determinations were made by the Hagedorn-Jensen method and glycogen determinations by Pflüger's method (micro method), the glucose being estimated after hydrolysis by the Folin-Wu or the Somogyi method. Desmo- and lyo-glycogen were determined by the method of Willstätter & Rohdewald [1934]. The glycogen values were calculated as glucose. For the determination of total acetone bodies in the urine Engfeld's method was used and for the nitrogen Kjeldahl's method (half micro method). The animals were killed by a blow on the neck and subsequent bleeding. The organs were cooled on a freezing mixture.

Results

We give in the first place the comparative values for blood sugar, for liver glycogen and muscle glycogen of rats which have fed on the various diets and which were killed without being made to fast (Table I).

Table I

No. of animals	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
10	70–140	116	4·11	0·32	20 p.c. casein or wheat
5	70–140	118	1·38	0·29	70 p.c. casein
5	70–140	112	1·12	0·30	90 p.c. ,,
5	70–140	120	1·42	0·22	Meat

Fasting experiments of 24 *and* 48 *hours*

We next carried out a series of experiments involving 24 and 48 hours' fast after natural and synthetic diets.

The results of these experiments are given in Tables II and III.

An experimental series after 12 to 15 hours' fast was then carried out to see whether the effect on the carbohydrate reserves of the liver of a diet high in protein appears after this shorter interval. The shorter the

TABLE II. 24 hours' fast

No. of animals	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
15	50–100	79	0·09	0·102	Wheat
15	50–100	116	1·32	0·215	Meat
9	70–120	100	0·069	0·078	20 p.c. casein
9	70–120	109	1·00	0·191	90 p.c. ,,
5	70–100	105	0·08	0·121	20 p.c. ,,
5	70–100	122	1·42	0·162	70 p.c. ,,

TABLE III. 48 hours' fast

No. of animals	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
12	60–200	77	0·11	0·072	Wheat
17	60–200	120	1·05	0·143	Meat
5	100–150	82	0·38	0·090	20 p.c. casein
5	100–150	124	1·42	0·12	70 p.c. ,,

fast the more we have to reckon with the error that arises from the fact that the last intake of food previous to the fast cannot be ascertained. But the results (Table IV) are qualitatively so similar in the individual experiments, and the differences in the average values are so great that the result appeared to us to be quite clear.

TABLE IV

No. of animals	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
7	60–100	98	0·64	0·164	20 p.c. casein
7	60–100	100	2·18	0·209	70 p.c. ,,

Phloridzin experiments

To induce still greater loss of sugar, we have produced a phloridzin diabetes during the fast by injecting phloridzin, dissolved in 2 p.c. soda solution, in a dose of 60–120 mg. per 100 g. animal daily in 2–3 single doses. The amount of sugar excreted during 24 hours in the urine (between 200 and 660 mg. sugar) varied according to the size of the animal and the dose of phloridzin. The results are given in Table V.

It must be remembered that these amounts of sugar are in some cases considerably greater than the total carbohydrate reserve of a well-nourished rat. It is important to note that in nearly all the experiments more sugar was excreted by the protein-fed animals than by those fed on carbohydrate. The animals were killed 14–18 hours after the last phloridzin injection; by this time the phloridzin action has already long ceased.

TABLE V

No. of animals	Weight g.	Blood sugar mg./ 100 c.c.	Sugar in urine in 24 hours mg.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
16	60–110	82	310	0·04	0·072	Carbohydrate-rich
18	60–110	120	400	0·53	0·124	Protein-rich

Half the number of animals were fed on wheat or on meat respectively, the other half on the diet with 20 p.c. casein or with 70 p.c. casein. The result for both series is practically the same.

It is important to note that after the final phloridzin injection a long time elapses before the animals are to be examined. If the rats are killed during the phloridzin action, that is 2–4 hours after the last injection, the differences described are absent or less marked (see below).

Effects of cold

We wished to see whether in spite of the heavy consumption of carbohydrate evoked by the influence of cold, differences could still be found in the carbohydrate reserves in protein- and carbohydrate-fed animals respectively. The animals were exposed for 5–7½ hours to a temperature of 6–12° C. Food was not provided during that time. As in the preceding experiment, some of the animals were fed on wheat or meat respectively, the majority on the 20 p.c. or the 70 p.c. casein diet. The results of the two series were the same as shown in Table VI.

TABLE VI

No. of animals	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
11	60–120	114	0·20	0·14	Carbohydrate-rich
11	60–120	133	1·02	0·19	Protein-rich

Work experiments

It was of special importance to examine how the carbohydrate reserves of the differently-fed animals would behave after a performance of hard work. The rats were put for a definite time (usually 1–2 hours) in a water-bath at 30° C. (room-temperature 18–20° C.), so they were compelled to execute swimming-movements all the time. Several series of experiments were carried out. In the first the animals (previously fasted for 2–12 hours) were killed immediately after the exercise. In the second, the animals (previously fasted 0–5 hours) were killed after resting 4–20 hours subsequent to the exercise. The results are given in Table VII.

TABLE VII

Series	No. of animals	Period fast hr.	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
Immediately after swimming:							
1	6	2–12	65–105	81	0·18	Trace	20 p.c. casein
1	6	2–12	65–105	86	0·045	Trace	70 p.c. ,,
1	6	2–12	75–100	82	0·058	0·032	70 p.c. ,,
1	5	2–12	80–125	91	0·108	0·01 (trace)	20 p.c. ,,
1	5	2–12	80–125	102	0·037	0·01 (trace)	70 p.c. ,,
After recovery:							
2	8	0–5	70–110	81	0·05	0·079	20 p.c. ,,
2	8	0–5	70–110	106	0·48	0·10	70 p.c. ,,
2	6	0–5	75–100	98	0·32	0·10	70 p.c. ,,
2	5	0–5	80–125	73	0·02	0·045	20 p.c. ,,
2	5	0–5	80–125	101	0·346	0·074	70 p.c. ,,

The data from all the swimming experiments may be summarized as follows:

	No. of animals	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
Before recovery	11	86	0·144	0·01	20 p.c. casein
After recovery	13	77	0·035	0·062	20 p.c. ,,
Before recovery	17	90	0·046	0·017	70 p.c. ,,
After recovery	19	101	0·38	0·090	70 p.c. ,,

The total result of the work-experiments shows that, under the conditions of work employed, the carbohydrate reserves dwindle to a considerable extent in animals on either diet, but that with those on a protein-rich diet, regeneration through glyconeogenesis in the recovery period is far more strongly marked than in the animals on a diet rich in carbohydrate; thus, with the former the glycogen content of the liver greatly increases, and the blood sugar rises, while with the latter the glycogen content of the liver falls further, and the blood sugar decreases. Only the muscle glycogen increases about equally in the two cases.

Experiments with killed bacteria (paratyphus B)

It is well known that by injecting certain bacterial products the glycogen of the liver can be reduced. This change keeps pace with changes of the blood sugar. Temperature changes usually accompany changes of metabolism. In order to see how differently-fed rats reacted to bacterial products, we injected 1 mg. of paratyphus B bacteria that had been killed and washed[1] into rats fed on a protein-rich diet, and into others fed on a diet rich in carbohydrate. The temperature sank in all the animals. The results, which are given in Table VIII, show that after

[1] We owe the preparation to Dr Olitzki.

6 hours the glycogen content of the liver had sunk to a small amount; the blood sugar showed no important changes. Twenty-four hours after the injection, there was a further fall in liver glycogen content and blood sugar of the animals on the carbohydrate-rich diet. In the protein-fed animals, on the contrary, the blood sugar remained at the normal height, and the liver glycogen increased quite considerably. Muscle glycogen decreased in both cases, perhaps somewhat less markedly in the protein-fed animals.

TABLE VIII

No. of animals	Hours after injection	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Diet
5	6	80–100	107	0·113	0·322	20 p.c. casein
5	6	80–100	105	0·078	0·329	70 p.c. „
5	24	80–106	82	0·026	0·128	20 p.c. „
5	24	80–106	106	0·377	0·144	70 p.c. „

Experiments with 1 : 2 : 5-*dinitrophenol*

Rats which had been fed with the 20 p.c. or the 70 p.c. casein diet received an injection of 2 mg. 1 : 2 : 5-dinitrophenol per 100 g. In all the experiments a large decrease in glycogen content and marked fluctuations of temperature were noticed. We examined nine pairs, the majority of 10 hours after the injection, the others 24 hours after it. In all cases the fall in glycogen was much less marked in the animals on a protein-rich diet than in those fed with abundance of carbohydrate.

Fasting experiments of 3 *days*

Now, if in the case of animals fed on a diet rich in protein and poor in carbohydrate the new formation of sugar was always so powerfully stimulated that even with the heaviest demand of the carbohydrate metabolism glycogen in the liver increased, in the case of a long fast, even after ordinary diet, an increase of glycogen would again be expected to occur. In fasting, the organism, after using up the carbohydrate reserves within the first day (in rats), lives on its stocks of protein and fat. It is thus using material relatively rich in protein and poor in carbohydrate. The results of the experiments with 3 days' fasting are given in Table IX, and should be compared with those in the case of 1 and 2 days' fast respectively (Tables II and III).

TABLE IX

Diet	No. of animals	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.	Total acetone mg.
20 p.c. casein	16	100–165	92	1·22	0·138	2·14
70 p.c. „	12	100–165	94	1·15	0·128	1·49

The nitrogen excretion was as follows:

Diet	1st day's fast mg.	2nd day's fast mg.	3rd day's fast mg.
20 p.c. casein	72·14	88·24	108·6
70 p.c. „	242·34	145·6	152·0

Note. In two experiments the weight of the rats was over 200 g.

It is very remarkable that on the third day of fasting, in the carbohydrate-fed animals, carbohydrate reserves reappear in considerable quantity. In the protein-fed animals we find on the average a similar amount. In the rats with 20 p.c. casein diet, there can be no doubt that on the third day of the fast, when the animals are usually in good condition, a glycogen synthesis sets in anew (in isolated cases this occurs as early as the second fast day). Since the carbohydrate reserves have been used up on the first fast day, we have with these animals an experiment analogous to one with a diet rich in protein, but the protein is taken from the animals' own substance. In this respect the rise in the nitrogen-values in the urine on the second, and still more on the third, fast day, is interesting. It is important to note further that the nitrogen excretion of the animals on the protein-rich diet is also far higher on the second and third fast days than that of carbohydrate fed animals—which points to a continuing increase of protein decomposition. It was surprising that the quantity of total acetone in the urine of the protein-fed animals was almost throughout lower than in the case of the carbohydrate-fed animals. A similar discovery has been made in hunger experiments on Eskimos, who also have a diet unusually rich in protein [Heinebecker, 1928; Rabinowitsch & Smith, 1936]. This is in accord with Mirsky's view [1936] that an increased formation of acetone bodies always takes place when the liver becomes poor in glycogen. It is precisely in the protein-fed animals that such impoverishment did not occur.

Effect of various diets

In order to find out whether the protein content of the food could be reduced below 70 p.c. and yet give the same effect, we administered other food mixtures containing smaller amounts of protein and noted whether in the fasting—or in the phloridzin experiment—a positive effect, i.e. a maintenance of glycogen reserves, was attained. These diets contained 36, 40, 45 and 50 p.c. of protein.

The results indicated that the effect of a protein-rich diet could still be obtained when the protein content of the food was 50 p.c. but not when it was lower than this.

The next question to be elucidated was, whether by a diet rich in fat but low in protein we could obtain an effect similar to that of a protein-rich diet. To test this a diet containing 50 p.c. fat, 10 p.c. casein and 32 p.c. carbohydrate was administered and a 24 hours' fasting experiment and the fasting-phloridzin experiment used (Table X).

TABLE X

No. of animals	Weight g.	Blood sugar mg./100 c.c.	Liver glycogen p.c.	Muscle glycogen p.c.
5	75–112	85	0·05	0·115

A diet rich in fat with a low protein content can therefore not provoke the effect described in the case of the protein-rich diet. We may therefore speak in future of a "protein effect".

When does this protein effect of the diet make its appearance, and when can it be made to vanish again? We have carried out a cross experiment of the following kind to find the answer to these questions. Two rats were fed on the 20 p.c. casein diet and two on the 70 p.c. After feeding for 12 days, we placed a rat having 20 p.c. casein for 2 days on 70 p.c. casein diet (designated 20/70 p.c.), and correspondingly transferred a rat on 70 p.c. casein for 1 day to 20 p.c. casein (designated 70/20 p.c.). Then we let the animals fast for 24 hours. The weight of the animals before the experiment came to 103–110 g. The result is shown in Table XI.

TABLE XI

	20 p.c. casein	20/70 p.c. casein	70 p c. casein	70/20 p.c. casein
Blood sugar, mg. p.c.	87	92	98	85
Liver glycogen, p.c.	0·078	1·35	1·00	0·072
Muscle glycogen, p.c.	0·21	0·265	0·143	0·130

Thus, 1 day of transference from a protein-rich diet to a diet rich in carbohydrate is sufficient to cause the protein effect to disappear, and on the other hand there suffices a 2 days' diet rich in protein, or, as we were able to ascertain, 1 day of fasting and 1 day of diet rich in protein after the diet rich in carbohydrate, to produce the protein effect of the diet.

We have not succeeded up to the present in producing the protein effect artificially by injecting amino-acids, peptones or proteins with simultaneous or previous fasting. We have not succeeded either in intensifying it, still less in evoking it artificially, by supplying certain

hormones.[1] On the other hand, it was found possible to suspend the protein effect. The following experiments were made on rats deprived of both adrenal bodies. Making an addition of 2 p.c. NaCl to the diet and supplying a 1 p.c. NaCl solution as drinking water, rats lacking adrenal bodies were fed either with meat only, or with a diet consisting of 90 p.c. casein and 10 p.c. fat. It was observed that these animals were not in a markedly worse condition than rats provided with the usual diet rich in carbohydrate. The blood sugar remained at the same level as in animals fed in the ordinary way. This is not easy to harmonize with the widely expressed view that the hormone of the adrenal cortex influences the transformation of protein into carbohydrate. But for our question it is an important fact that in three experiments with 16–24 hours' fasting in animals on a protein-rich diet, we found only traces of glycogen left in the liver, and the blood sugar fell, while after removal of only one adrenal body liver glycogen was found as usual. Thus after extirpation of the adrenal bodies, the protein effect no longer makes its appearance. We cannot yet be certain whether the endogenous formation of new glycogen was hindered or whether according to the Fisher *et al.* [1936] view of hypophysis action we could conclude that the carbohydrate consumption in these animals when fasting increases, rather than decreases as in normal animals. In hyperthyroidized rats, too, the protein effect was absent. With these animals we have injected 0·3 mg. thyroxin per 100 g. of animal, 4 days before the examination. It is probable here that with the intensified metabolism, glycogen is indeed formed afresh, but is immediately consumed; the action therefore would be secondary.

The fundamental researches of Willstätter & Rohdewald [1934] have opened up new possibilities for the eventual explanation of the varying relation between glycogen and glycogenase in the cell. They have shown that glycogen and diastase occur in various forms in the cell, and the conceptions desmo- and lyo-diastase, desmo- and lyo-glycogen and glycogen-simplex were coined for the various forms. We have, therefore, made experiments to test the possibility that our findings could be explained by quantitative differences in the different forms.

At first, in addition to the total glycogen, we determined regularly in all experiments desmoglycogen and lyoglycogen, according to Willstätter & Rohdewald's method [1934]. But we were unable to

[1] Acetone-dried preparation of the anterior hypophyseal lobe (I.G. Farbwerke) in *p*H 7·4 phosphate buffer extracted and Eschatin, a preparation from the cortical part of the adrenal gland, have been administered.

find any characteristic changes due to diet or to the various methods of arranging the experiments. At first it was thought that the glycogen simplex, determined by Willstätter's method, was different in composition, in "protein" and "carbohydrate" animals (experimental animals—dogs and cats), the protein content being higher, and the glycogen content lower in the "protein" animals. If, however, the glycogen content in the livers was about the same the difference just described, although present, was comparatively small (Table XII). The

TABLE XII

Animal	Glycogen in the liver p.c.	Glycogen in the simplex p.c.	Protein in the simplex p.c.	Food
Dog	2·8	41·8	38·92	Meat
,,	2·83	34·13	45·83	,,
,,	2·8	47·0	37·86	,,
,,	5·0	78·52	17·83	Rich in carbohydrates
,,	5·0	66·43	22·82	,, ,,
Cat	4·35	46·4	32·21	Meat
,,	4·7	39·49	38·66	,,
,,	4·76	51·35	30·9	Rich in carbohydrates

diastatic action of the liver in the differently-fed animals, examined according to Scharles *et al.* [1934], yielded no differences. No examination according to the new principles of Willstätter & Rohdewald [1937] has yet been made. It likewise remains for us to investigate whether another chemical form of glycogen is present, as has been described recently by Bell [1936] in connexion with Haworth's researches.

DISCUSSION

We have now to consider why very large carbohydrate reserves in the liver of the normal animals on a diet rich in carbohydrate, with all possible demands on the carbohydrate metabolism, disappear so extremely fast and the much smaller reserves in the protein-fed animals are so relatively well maintained.

The following possibilities may be considered. First, a fundamentally different speed of absorption in the case of the different forms of diet. There is no support for this. Second, it might be that with the protein-fed animals protein reserves are laid down in large amounts in the liver and that these reserves could be exploited with particular ease for the building up of fresh carbohydrate. We have to-day very good reason to believe that such protein reserves exist in the liver [Luck, 1936]. But they can scarcely be brought forward in explanation of the protein effect, since the fresh formation of glycogen on the third fast day, where there

has been a previous diet rich in carbohydrate, cannot be explained in this way. Again, the cross experiment introduced by us refutes such an explanation. If for 1 day we transfer rats that were fed with 70 p.c. casein to 20 p.c. casein, the protein effect is no longer demonstrable. It is unlikely that with this procedure the protein reserves in the liver should have disappeared in 1 day, especially as there is considerable protein present even in the later diet. Accordingly, so far as we see, there remain only two possible explanations: (*a*) the glycogen or glycogenase, or both together, have so altered in the "protein" animals, or are present in such a form that exhaustion of the glycogen in the liver can proceed only at a much slower rate, or is even impossible. We have an apparent example of such an occurrence in the so-called "glycogen disease" of v. Gierke; in this disease the liver is loaded with glycogen, but the exhaustion of it has apparently become impossible. We recall further the experiments of Fluch *et al.* [1934], who found, when perfusing the livers of frogs deprived of their hypophysis, that less sugar appears in the perfusate than in the case of control animals with a similar glycogen content in the liver. (*b*) The formation of fresh sugar is so much greater in the protein-fed animals than in those fed on carbohydrate that no matter how heavy the demand, fresh gluconeogenesis can always occur.

It is certain that the glycogen of the protein animals with a heavier demand of the carbohydrate metabolism can first of all vanish to a minimal amount. That is shown by experiments after performance of heavy work, experiments after the action of bacterial products, the action of thyroxin, adrenalin and dinitrophenol.

On the ground of the experiments described (*a*) cannot yet be brought forward for the explanation of the protein effect. On the other hand, a considerable amount of evidence for (*b*) may be given. This may be summarized as follows:

(1) In the swimming experiments, the glycogen disappears in both protein- and carbohydrate-fed animals after the work. After the rest period, a marked increase of the glycogen in the liver is to be noticed only in protein-fed animals. With carbohydrate-fed animals the glycogen decreases further.

(2) After injection of bacterial substances (paratyphus B), the temperature falls at the beginning, and the glycogen in the liver of protein-fed and carbohydrate-fed animals falls off 24 hours after the injection. Later, in the carbohydrate-fed animals the glycogen decreases further, in protein-fed animals a remarkable rise ensues.

(3) If after phloridzin action the animals are not examined until 14–20 hours have elapsed, we find in the protein-fed animals a great quantity of glycogen in the liver, notwithstanding the heavy loss of carbohydrate (diabetes). In the carbohydrate-fed animals glycogen is not found. This, notwithstanding that in the protein-fed animals the sugar excretion is usually greater than in the carbohydrate-fed animals.

(4) On the third day of the fast we find in rats fed on a diet rich in carbohydrate a glycogen content of the liver again averaging over 1 p.c. The formation of fresh glycogen commences when the rats begin to use endogenous protein.

(5) We have instituted continuous experiments on mice, one group fed with meat and the other with wheat, afterwards giving them a varying fasting period. The experiments appear to indicate that in the animals previously fed with meat, during the fast such a vigorous new formation of carbohydrates sets in, that after long fasting periods more glycogen was often found in the liver than at the beginning of the experiment, or after a short fast.

Room temperature, 24° C.

	Wheat		Meat	
	Liver glycogen p.c.	Muscle glycogen p.c.	Liver glycogen p.c.	Muscle glycogen p.c.
Before fast	1·48	0·109	0·418	0·064
7½ hours' fast	0·043	0·057	0·460	0·058
24 „	1·53	0·105	1·39	0·049

Accordingly, we feel that we must explain all the experiments described by postulating that intensified glyconeogenesis is the cause of the maintenance of the carbohydrate reserves in protein-fed animals, even in cases where, as during a fast or under the influence of cold, the glycogen does not seem to decrease at all. Where the glycogen, having dwindled, reappears after a rest period, the process is self-explained as glyconeogenesis. Thus, by a preponderance of the protein metabolism, an endogenous glyconeogenesis is provoked, which, even under the heaviest demands on the liver glycogen is so powerful that not only is the blood sugar regenerated, but liver glycogen is formed anew. Thus it seems that an excessive decomposition of protein is the strongest stimulus for a continuous glyconeogenesis in the liver. Whether this stimulus acts directly on the liver, or indirectly through certain hormones has not yet been made clear. It must be borne in mind that at the same time a

diminished carbohydrate combustion is bound up with this process. The making of such an assumption for the fast of long duration is obvious. But the new experiments of Soskin & Mirsky [1935] support the view that there is absolutely no decrease in the consumption of sugar in fasting. The symptoms of hunger diabetes can be traced to an increased glyconeogenesis. It is certain that when much protein is being metabolized carbohydrate metabolism takes different lines from those which it follows when it is itself the chief fuel.

The protein effect described might be of importance for the pathology of carbohydrate metabolism, and also for the physiology of work. We know already that heavy work can be performed better on a protein-rich diet than on one rich in carbohydrate [Wishart, 1934]. Our own experiments also give support to the significance of a diet of this kind under conditions of cold.

It is clear that to prescribe a diet rich in protein or one rich in carbohydrate is not merely a matter of calculating calories. The reaction of the metabolism to various increases in demand, such as those caused by hunger, cold, work, poisons, etc., can change in a radical fashion with the alteration of the diet.

Summary

1. The high glycogen reserves, especially in the liver, with the normal feeding of rats on a diet rich in carbohydrate (in our cases about 4 p.c. glycogen) are brought easily almost to vanishing point by increased demands of various kinds on the carbohydrate metabolism. The far smaller glycogen content in the case of excessive protein-feeding (1·4 p.c. glycogen in the liver) is little affected by the same forms of demand.

(*a*) After 24 or 48 hours' fast, where there has been previous excess of carbohydrate in the diet, only vestiges of glycogen are found, but in the protein-fed animals in all the experiments taken together, round about 1·2 p.c.

(*b*) With 24 hours' fast and simultaneous administration of phloridzin, it is found that although the sugar excretion in the urine is mostly greater than the amount of the total carbohydrate reserves, in the protein-fed animals amounts of 0·5 p.c. glycogen can still be found in the liver, while with the carbohydrate-fed animals usually only minimal amounts are present.

(*c*) After an average of 5 hours' action of cold at a mean of 10° C., the glycogen almost vanishes in the carbohydrate-fed animals, whereas in the protein-fed animals a mean of 1 p.c. glycogen is found.

2. After 3 days' fasting, newly formed glycogen (on the average 1·2 p.c.) is found in the liver of the carbohydrate-fed animals and a like amount in the protein-fed animals.

In the fasting experiments muscle glycogen and blood sugar show differences qualitatively similar to those for the liver glycogen, but quantitatively much smaller.

3. After swimming experiments, the glycogen disappears to a great extent in both series of animals. After a subsequent rest pause of 4–24 hours without giving food, the glycogen in the liver decreases still further in the carbohydrate-fed animals, but in the protein-fed animals a pronounced fresh building up of glycogen in the liver is observed.

Similar results were observed after injecting minimal amounts of dead bacteria (paratyphus B).

4. In animals fed with an excess of fat and with the usual protein content of the diet, a maintenance of the glycogen reserves was not observed. The actions described must therefore be connected with the disintegration of protein (protein effect).

5. After removal of the adrenal bodies, the protein effect no longer supervenes.

6. It is concluded that an intensified glyconeogenesis is the most important cause of the phenomena described. Excessive katabolism of protein leads to a powerful incitement to glyconeogenesis, so that even in starvation and in heavy claims on the carbohydrate metabolism glycogen is freshly formed in the liver.

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THE EFFECT OF THE INTERACTION OF IONS, DRUGS AND ELECTRICAL STIMULATION AS INDICATED BY CONTRACTION OF THE ANTERIOR RETRACTOR OF THE BYSSUS OF *MYTILUS EDULIS*

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Winton [1937] has described the results of stimulating this plain muscle with alternating current (A.C.) and direct current (D.C.). The present series of observations is concerned with describing the properties of the contraction produced by these and various other agents, with a view to attempting an analysis of the interrelation between ions, drugs and electrical stimulation in respect of their potentiating or antagonizing actions. The significance of the relations discovered in connexion with contraction has been extended by a preliminary analysis of the effects of the relevant agents on the water and total base content of the muscle [Singh, 1938].

Methods

The standard experimental solution, referred to below as *Mytilus* saline, had a composition of 1·8 c.c. 0·564*M* KCl, 2·8 c.c. 0·376*M* $CaCl_2$, 5 c.c. *M*/15 sodium phosphate at *p*H 7 with the requisite amount of sodium chloride to make it isotonic, 0·564*M* NaCl being added to bring the total volume up to 100 c.c.

The muscle was electrically stimulated by Winton's method [1926], modified by including a 12 μF. condenser in the circuit to obviate rectification at the electrodes when A.C. was used. Variations of excitability to A.C. or D.C. were examined by two methods: first, the voltage of a stimulus of 10–15 sec. duration needed to evoke a small tension of a definite value was determined before and after immersing the muscle in the test solution for 15 min.; secondly, the tension evoked by a stimulus of given strength (8–10 V., 10–15 sec.) applied to the muscle immersed in different media was employed as an indication of the varying sensitivity of the muscle;

the two methods invariably gave the same results. The sensitivity to stimulation by ions was usually measured in terms of the tension evoked by a given concentration of the substance, and occasionally in terms of the concentration needed to evoke a given small tension.

Results

Electrical stimulation. During continued stimulation with A.C., the tension usually subsides to zero and remains so whether the current continues for 5 min. or 2 hours. This is due to a rise in threshold, as shown by the fact that if the initial stimulus is 8 V., the muscle contracts again if the voltage is increased suddenly to 16 V., and then again if further increased to 24 V. (Fig. 1). When the current is stopped or suddenly

Fig. 1. Continuous stimulation with A.C.

reduced, there is usually a contraction at each successive reduction (the off-contracture). Rarely during the passage of A.C., a continuous tension, as in the tetanus of frog muscle, is produced; or the tension rises again after subsiding or the muscle contracts and relaxes rhythmically.

Chemical stimulation. The muscle may be stimulated (1) if the sodium of the *Mytilus* saline is partly replaced by equivalent amounts of other cations, such as potassium (usually over 0·05 *M*); the divalent cations, calcium (over 0·075 *M*), strontium (over 0·075 *M*), barium (over 0·01 *M*), their stimulating power varying in the order Ca < Sr < Ba; (2) if the chloride is partly or wholly replaced by other anions, such as bromide, nitrate, iodide, thiocyanate, cyanide, sulphate, citrate, the stimulating power of some of these solutions being in the order

$$NaCl < NaBr < NaNO_3 < NaI < NaSCN < NaCN.$$

The cyanide ion is a very powerful stimulant, being sometimes effective in concentrations of less than 1 in 10,000 NaCN; (3) if certain drugs are added to the *Mytilus* saline, such as adrenaline (usually over 1 in 10^6), acetylcholine (usually over 1 in 10^6), veratrine (over 1 in 1000), caffeine (over 1 in 1000), trimethylamine (over 1 in 10,000), bile salts. Ether

dissolved in *Mytilus* saline sometimes produces contraction. A feeble contracture may be produced if the sodium chloride of the *Mytilus* saline is replaced with osmotically equivalent amount of glucose. Rarely urea (5–10 p.c.) in *Mytilus* saline may produce a feeble contracture, or after treatment with urea tone may be greatly increased. In the experiments described below the standard chemical stimulus used has been potassium (from 0·05 to 0·1 *M*).

As with electrical stimulation, the tension produced when excess of potassium is added subsides, though potassium is still present in the solution. This is due to a rise in threshold, for sudden successive increases in the concentrations of potassium again produce corresponding successive contractions. Akin to the contracture produced on the cessation of the A.C. stimulus, a contraction may result on cessation of the chemical stimulus, that is, when the stimulating chemical is withdrawn. This occasionally occurs with adrenaline (1 in 50,000), acetylcholine (1 in 50,000), veratrine hydrochloride (1 in 1000), and sodium sulphate (0·56 *M*). As with A.C. stimulation, the muscle may contract and relax rhythmically when it is immersed in some of the chemical stimulants mentioned above (0·056 *M* KCl, 0·037 *M* $BaCl_2$, 0·564 *M* NaCl, NaBr and $NaNO_3$, 0·033 *M* NH_4Cl, 1 in 1000 caffeine).

The excitability to electrical and chemical stimulation was different at different times of the year. During summer the muscle was hyperexcitable to A.C. and relatively inexcitable to potassium and other chemical stimulants. During winter the muscle was hyperexcitable to potassium and less excitable to A.C. or D.C. In summer, fatigue was rapid when the muscle was stimulated with potassium. The potassium contraction exhibits well-marked beneficial effect of previous contraction (staircase phenomenon). This fact was often utilized to remove inexcitability to a smaller dose by preliminary treatment with a larger dose.

The muscle can also be stimulated mechanically or by a sudden change of osmotic pressure. Sometimes, in winter, it contracts spontaneously when immersed in sea-water or *Mytilus* saline.

Properties of the A.C., D.C. and the potassium contractions

Effect of potassium and ammonium. The optimum concentration of potassium necessary for the A.C. contraction is twice that of sea-water or the same as that of *Mytilus* blood (0·020 *M* KCl). Sudden increase in the concentration of potassium or ammonium (in acid solutions at *p*H 7, i.e.

effect of NH_4^+) in *Mytilus* saline by partial replacement of the sodium depresses the excitability to A.C.; this is followed by an increase in excitability (Fig. 2), which lasts for a considerable period ranging from a few minutes to a couple of hours, after which the muscle gradually becomes inexcitable. These effects of ammonium and potassium on *Mytilus* muscle resemble the effects of ammonium on frog muscle nerve [Ing & Wright, 1931]. The stage of initial depression can be avoided if the concentration of potassium is increased slowly. In winter, when the muscle is hyperexcitable to potassium and less excitable to A.C., there is only a depression in excitability to A.C., whether the concentration of potassium is increased slowly or suddenly. These differences between the summer and winter muscles can be artificially reproduced by variations of the calcium content (0·0–0·02*M* $CaCl_2$) of the *Mytilus* saline. With a low calcium content there is a depression of excitability, the secondary rise being absent, the muscle becoming inexcitable in a few minutes. Calcium also hastens the recovery from the depressant effect of excess of potassium.

Further the depressant effect of potassium on the excitability to A.C., besides being intensified by lessening of the calcium content of *Mytilus* saline, can be increased if larger concentrations of potassium are used. The depressant effect of potassium is greater than that of ammonium, and if small concentrations of these ions are used (0·05*M*) to avoid the depressant effect, the secondary potentiating effect of potassium on the excitability to A.C. is greater than that of ammonium.

When the potassium-rich or ammonium-rich solution is replaced with *Mytilus* saline, the restoration of the initial excitability to A.C. is preceded by a stage of hyperexcitability (Fig. 2). Again the effect varies with the season and individual muscles. If the muscle is highly sensitive to potassium, then the depression of excitability may be permanent, or the stage of hyperexcitability during recovery may be absent. These variations can be artificially reproduced by altering the calcium content of *Mytilus* saline, or by varying the concentration of potassium, the depressant effect being greater if large concentrations are used (0·1–0·2*M* KCl).

The effect of cations on the potassium contraction are, in general, opposite to those on the A.C. contraction. Ammonium chloride at first raises the excitability to potassium and then abolishes it (Fig. 3). This is the only method known which will render a muscle inexcitable to potassium and hyperexcitable to A.C. If the ammonium chloride be added slowly enough, the phase of hyperexcitability to potassium disappears, as does the phase of hyperexcitability to A.C. Treated with potassium

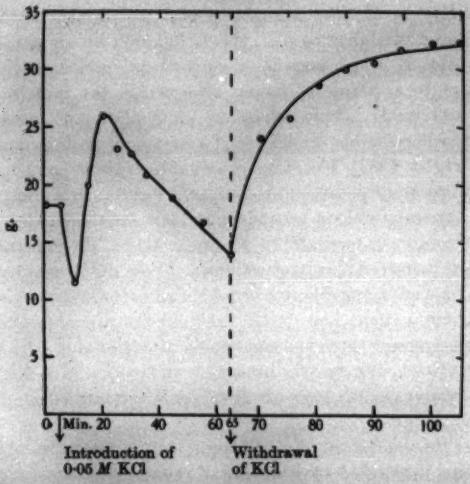

Fig. 2. Effect of potassium on tension produced by A.C. (7 V.–10 sec.) in a summer muscle (June 1936). A steady state was at first produced in *Mytilus* saline; the latter was then replaced with *Mytilus* saline, the sodium chloride of which had been partly replaced with potassium chloride (0·05 *M*). The latter solution was then again replaced with *Mytilus* saline.

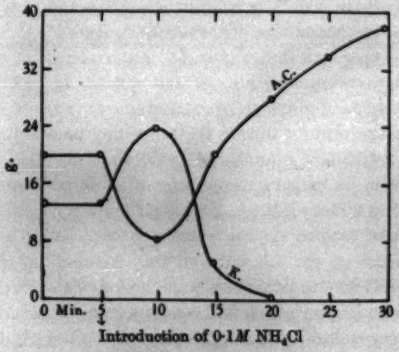

Fig. 3. Effect of ammonium on tension produced by A.C. (8 V.–10 sec.) and potassium (0·1 *M* KCl) in a summer muscle.

itself a muscle similarly becomes inexcitable to potassium. The initial sensitizing and the subsequent densitizing effect of potassium is greater than that of ammonium.

Effect of sodium. (*a*) *Addition*: The effects of addition of sodium chloride to *Mytilus* saline or sea-water are complicated by the inevitable increase in osmotic pressure, nevertheless its addition, if performed gradually, yields changes in excitability like those consequent on sudden increase in the concentration of potassium or ammonium. Gradual increase in the concentration of sodium chloride may at first depress and then increase the excitability to A.C. or, as with gradual increase in the concentration of potassium or ammonium, the initial stage of depression may be absent. Sudden addition of excess of sodium chloride only depresses the excitability to A.C., the subsequent increase being absent; the excitability to potassium is at first raised and then depressed. Control experiments in which the osmotic pressure is increased by the addition of glucose only show a depression in excitability, both to A.C. and to potassium. (*b*) *Withdrawal*: This was done in two ways. The muscle was placed either in a hypotonic solution, or in a solution in which part of the sodium chloride had been replaced with an osmotically equivalent amount of glucose. The first effect of the diminution of the sodium content is to increase the excitability to A.C. in a way comparable to the effect of withdrawal of ammonium or potassium; this phase of increased excitability is followed by one in which the excitability is depressed. These results are more often obtained if the sodium is withdrawn gradually than if it is withdrawn suddenly. The phase of increased excitability on withdrawal of sodium is sometimes transient or absent. It is difficult to say how far the subsequent decrease in excitability to A.C. is due to decrease in the conductivity of the solution. Ciliary movement in *Mytilus edulis* continues for several hours if the sodium chloride of the surrounding medium is wholly replaced with saccharose [Gray, 1922].

Effect of withdrawal of sodium on the excitability to potassium depends upon whether the sodium chloride is replaced with glucose or not. The effect of hypotonic saline (60–70 p.c. of normal) is the opposite to that of *Mytilus* saline rendered hypertonic by addition of sodium chloride; the excitability is at first depressed and then raised. Hypotonic solutions produce a comparable increase in the sensitivity to drugs of the guinea-pig uterus [Dale, 1913]. If the sodium chloride is replaced with an equivalent amount of glucose the excitability to potassium is depressed; replacement of all the sodium chloride of the *Mytilus* saline with glucose renders the muscle inexcitable to potassium.

Effect of lithium. Replacement of the sodium of the *Mytilus* saline with lithium decreases the excitability to A.C. and increases that to potassium. If the depressant effect of the monovalent cations is avoided by using small concentrations (0·05*M*), they increase the excitability to A.C. and decrease that to potassium in the order $Li < Na < NH_4 < K$, being the same as the order for favouring ciliary movement in *Mytilus edulis* [Lillie, 1906]. The initial effect of lithium is sometimes in the opposite direction, the excitability to A.C. being increased and that to potassium

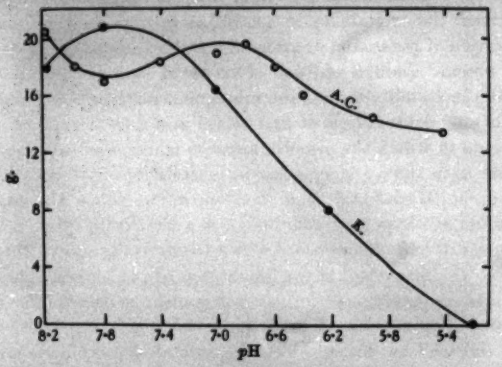

Fig. 4. Effect of *p*H on tension produced by potassium (0·1*M*) and A.C. (10 V.–10 sec.).

decreased; the monovalent cations then decrease the excitability to A.C. and increase that to potassium in the same order; subsequently the effect is reversed. Ultimately the excitability to A.C. is depressed by these abnormal cations in the order $Na < Li < NH_4 < K$; they also depress muscular activity in some other marine animals in the same order [Lillie, 1909].

Effect of hydrogen ion. The effects of this apparently resemble the effects of other monovalent cations, especially sodium. In six winter muscles the maximum excitability to potassium was at *p*H 7·8 (borate buffer *M*/300, phosphate *M*/300, bicarbonate as in sea-water). It diminished with change on either side, the muscle becoming inexcitable between *p*H 6·5 and 5 (Fig. 4). In five muscles (excitable to potassium) the maximum excitability to A.C. was at *p*H 8·2–8·4, that of aerated sea-water. As the *p*H was lowered, the excitability to A.C. declined and rose again to a second maximum at about *p*H 7, the intervening minimum

being at *p*H 7·8. With further lowering of *p*H, the excitability to A.C. declined, till the muscle became inexcitable at *p*H 5 or lower (buffer phosphate, acetate *M*/300). If carbon dioxide is used, inexcitability both to A.C. and potassium is rapid. Thus over the physiologically significant range (*p*H 8·4–6·8), the effect of *p*H follows the above-mentioned rule that when the excitability to potassium is highest, that to A.C. is lowest.

Sudden increase in the concentration of hydrogen ions produces effects similar to those consequent on sudden increase in the concentration of ammonium, potassium or sodium ions. If the *p*H is increased to

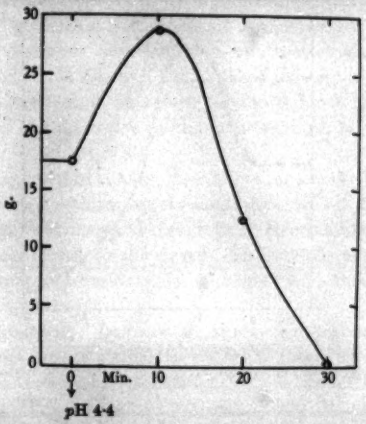

Fig. 5. Effect of sudden increase of [H⁺] on tension produced by potassium (0·1*M* KCl).

4·4, there is at first a great increase in the excitability to potassium, which is followed by a decline till the muscle becomes inexcitable (Fig. 5). As with ammonium or potassium, if the *p*H is gradually lowered to 4·4, this phase of increased excitability is absent. Thus there is antagonism between all the monovalent cations, not excluding the hydrogen ion. These effects of hydrogen ion can be understood when it is remembered that hydrogen ion, like sodium or potassium, can also make the muscle contract.

Effect of calcium. The optimum concentration of calcium necessary for the A.C. contraction is twice that of sea-water, or the same as that of *Mytilus* blood (0·021*M* $CaCl_2$). The optimum concentration of calcium for the potassium contraction is lower, being the same as that of sea-water. Excess of calcium depresses the excitability to A.C. whether the

concentration is increased gradually or suddenly; the muscle may, however, make a partial recovery. The depressant action of excess of calcium on excitability to A.C. is greater in winter potassium sensitive muscles. When calcium is excluded, the excitability to A.C. is depressed, but here again the depressant action of calcium deficiency varies with the sensitivity of the muscle. A muscle highly excitable to potassium became inexcitable to A.C. in a few minutes, but a muscle inexcitable to potassium (0·1*M* KCl) still responded to A.C. after 2 hours' immersion in calcium-free saline. When calcium is excluded the excitability to potassium at first rises and then falls (Fig. 6).

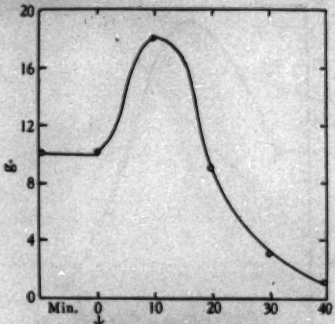

Fig. 6. Effect of withdrawal of calcium on tension produced by potassium (0·1*M* KCl).

Thus calcium deficiency depresses the excitability to A.C., just as does an excess of calcium or potassium which, if sufficient in amount, will stimulate the muscle. Moreover, removal of calcium from the medium elicits a contraction in those muscles which are highly sensitive to potassium, and such a contraction is presumably due to an exciting action of sodium, comparable to similar action of potassium, since it occurs in the absence of all other relevant ions, viz. potassium and magnesium. Moreover, if the muscle is highly sensitive to potassium, even *Mytilus* saline may elicit a contraction, which is abolished if the calcium content is doubled (0·02*M* $CaCl_2$). Calcium thus varies only the sensitivity of the muscle to sodium, just as it varies the sensitivity to potassium. Sodium, unantagonized by calcium, may therefore be regarded as the agent responsible both for stimulating the muscle and for depressing the excitability to A.C. when calcium is withdrawn. This view is supported by the restoration of excitability to A.C. in the absence of

calcium, which is sometimes produced by immersion of the muscle in sodium-deficient *Mytilus* saline (one in which 30–40 p.c. of the sodium chloride is replaced by an osmotically equivalent amount of glucose); similar treatment, moreover, has an antagonistic action on the production of contracture by withdrawal of calcium.

Diminished excitability to potassium in the absence of calcium would then be due to the antagonism of sodium, comparable to the antagonism between ammonium and potassium, or excess sodium and potassium. Treated with potassium itself a muscle similarly becomes inexcitable to potassium, the tension produced by a given concentration of potassium subsides though potassium is still present in the solution. A greater concentration of potassium now evokes a contraction showing that the subsidence of tension is due to a rise in threshold to potassium. Accommodation to potassium is therefore produced by a process which is probably similar to that which produces antagonism between the monovalent cations.

A muscle immersed in calcium-free saline for about an hour and then treated with isotonic sodium citrate solution for about 15 min. becomes inexcitable to A.C. and chemical stimulation. Excitability or contractility is not entirely abolished, as the muscle can be made to contract by immersion in isotonic sodium cyanide, combined with the cessation of the passage of a direct current.

Effect of strontium. Increase in the concentration of strontium chloride produces effects similar to those produced by increase in concentration of ammonium or potassium, that is, an initial depression, a subsequent rise, and a stage of hyperexcitability on removal. As with other ions the effects vary with individual muscles and with the concentration of strontium employed. The stage of initial depression is avoided by gradual increase in the concentration of strontium. The effects are obtained less readily with strontium than with ammonium or potassium.

Effect of magnesium. In winter potassium sensitive muscles magnesium depresses the excitability to A.C. in all concentrations; in summer, potassium insensitive, muscles the optimum concentration is one-third that of sea-water or that of blood. The optimum concentration for the potassium contraction is the same as that of blood. Sudden increase in the concentration of magnesium only produces a depression in excitability to A.C., sometimes with a partial recovery. An interesting feature is that excess of magnesium may prolong the latent period of the A.C. contraction to as much as 5 sec. (stimulus A.C. 10 V.–10 sec.).

Effect of barium. Barium increases the excitability to A.C. It has no depressant action, except when it causes the muscle to contract; the viscosity then becomes very high, and the muscle is rendered inexcitable to all forms of stimulation. In summer, potassium insensitive, muscles the divalent ions increase the excitability to A.C. in the order Ca < Sr < Ba. Summer muscles respond to A.C. even if they are immersed in isotonic solutions of calcium or strontium chlorides for about an hour, the muscles being insensitive to these cations; in barium chloride, however, the muscle contracts before it can be stimulated with A.C.

The optimum concentrations of calcium, potassium and magnesium for the D.C. contraction are the same as those for the A.C. contraction. Withdrawal of calcium causes the loss of excitability to A.C. more rapidly than to D.C., so that a muscle rendered inexcitable to A.C. may still respond to D.C.; the muscle still responds to the cessation of D.C. after the passage of the current has become ineffective.

Effect of anions. Excess of abnormal anions mentioned above depresses the excitability to A.C. and D.C., but increases that to potassium. The depressant effect of these anions on the excitability to A.C., and the sensitizing effect on the excitability to potassium varies in the order NaCl < NaBr < $NaNO_3$ < NaI < NaSCN < NaCN (Table I). The sensitizing

TABLE I. Effect of anions on excitability to A.C. and to potassium chloride of the *Mytilus* saline completely replaced with the anion. (Figures show g. tension.)

No. of Muscle	A.C.					Potassium				
	Cl	Br	NO_3	I	CNS	Cl	Br	NO_3	I	CNS
1	24	20	11	2	0	3	10	7	32	36
2	29	24	17	2	0	22	16	15	36	39
3	57	56	55	32	22	17	24	26	32	36
4	37	26	18	10	4	13	20	28	34	38
5	18	16	9	0	0	24	30	25	36	39
6	—	—	—	—	—	8	12	14	24	30
7	—	—	—	—	—	12	16	18	30	36
Average	33	28	22	9	5	13	18	19	32	36

effect of these anions (excluding cyanide) on the excitability of frog sartorius to chemical stimulation [Lillie, 1910; Chao, 1934*a*] and to stimulation by cold [Chao, 1934*b*], and the depressant effect on ciliary movement in *Mytilus* [Lillie, 1906] vary in the same order. The effect of nitrate is approximately the same as that of bromide (cf. the effect of bromide and nitrate on swelling of *Mytilus* muscle [Singh, 1938]); sometimes its potentiating effect on the excitability to potassium is less than that of bromide. This appears to be due to the great increase in viscosity caused by nitrate. If the muscle is immersed in *Mytilus*

saline, the chloride of which is replaced by nitrate, stimulation by potassium causes a great increase in viscosity, as judged by the slowness with which the tension increases and subsides; the muscle may now become inexcitable to all forms of stimulation, and take about a couple of hours to recover. Excess of sodium chloride and sodium bromide may produce a similar effect, but are much less potent than sodium nitrate. The depressant effect of nitrate on ciliary movement may be less than that of bromide [Lillie, 1909].

The effect of small concentrations (0·05–0·1*M*) and the initial effect of large concentrations, however, is in the opposite direction, that is, to increase the excitability to A.C. This power of increasing the excitability to A.C. also varies in the same order as above, cyanide being effective in concentrations of 1 in 200,000. The sensitivity of frog sartorius to electrical stimulation is increased by these anions (with the exception of cyanide) in the same order [Chao, 1935]. As with cations, the depressant effect on excitability to A.C. (1) predominates in winter, potassium sensitive, muscles, (2) can be accentuated by deficiency of calcium, (3) is accentuated by increase in concentration, so that the preliminary phase of hyperexcitability to A.C. may be absent. This is more likely to happen with anions whose depressant action is greater, such as iodide, than with anions whose depressant action is less, such as bromide.

The effects of cyanide in some respects differ from those of the other anions. *Mytilus* muscle is capable of reacting even in high concentrations of cyanide; it will respond to stimulation with D.C. even in isotonic solutions of sodium cyanide [Singh, 1937]. In intermediate concentrations (over 1 in 10,000) the muscle becomes more sensitive to A.C. and D.C., as well as chemical and mechanical stimulation. This unusual feature, namely a change in the same direction in excitability to A.C. and to potassium, appears to be in some way related to very high sensitivity of the muscle to chemical stimulation, as this effect is also produced by barium salts and the muscle is very sensitive to barium and cyanide.

Effect of drugs and other agents. The effects of certain drugs resemble the effects of the anions. A selection of typical results is given in Table II. The following drugs greatly decreased the excitability to A.C. (sometimes rendering the muscle inexcitable) and increased the excitability to potassium: adrenaline, acetylcholine, veratrine, strychnine, curare, physostigmine, novocaine and nicotine. As with anions, the effect of small concentrations and the preliminary effect of large concentrations of adrenaline, veratrine and caffeine was in the opposite direction, that is, to increase the excitability to A.C. The depressant effect of these three

TABLE II. Effect of certain drugs on the excitability to A.C. and to potassium.

Drug	Concentration	No. of muscle	Grams tension: A.C. 8 V.–10 sec. Before immersion	A.C. 8 V.–10 sec. Immersion for 10–15 min.	A.C. 8 V.–10 sec. Immersion for 1 hr.	K Before immersion	K Immersion for 10–15 min.	K Immersion for 1 hr.	Season
Adrenaline	1 in 6×10^6	1	30	36	—	12	6	—	Winter
	1 in 10^5	2	12	3	—	12	20	—	Winter
		3	32	36	—	0	0	—	Summer
Veratrine hydrochlor.	Saturated in *Mytilus* saline	1	15	0	—	7	21	—	Winter
		2	15	12	0	0	—	20	Summer
Caffeine	Saturated in *Mytilus* saline	1	26	0	—	10	28	—	Winter
		2	26	36	0	0	—	20	Summer
Acetylcholine	1 in 25,000	1	25	2	—	10	28	—	Summer
Strychnine hydrochlor.	1 in 1000	1	26	2	—	12	24	—	Summer
Curare	1 in 1000	1	16	6	—	0	16	—	Summer
Nicotine	1 in 100	1	26	4	—	0	25	—	Summer
Novocaine	1 in 400	1	26	2	—	3	18	—	Summer
Ephedrine hydrochlor.	1 in 400	1	20	2	—	2	10	—	Summer
Ether	0·5 p.c.	1	20	2	—	12	14	—	Summer
Chloral hydrate	1 p.c.	1	12	7	—	3	10	—	Summer

drugs on the excitability to A.C. (1) predominates in winter when the muscle is excitable to potassium, (2) can be accentuated by deficiency of calcium, (3) is accentuated by increase in concentration, so that the preliminary phase of hyperexcitability to A.C. may be absent; but, as with cations, the muscle sometimes makes a partial recovery from the depressant effect.

In sensitive winter muscles the concentration of adrenaline required to increase the sensitivity to A.C. and decrease that to potassium is about 1 in 6×10^6, while concentrations larger than 1 in 10^6 have opposite effects, 1 in 25,000 rendering the muscle inexcitable to A.C. and hyperexcitable to potassium. In summer, when the muscles are inexcitable to potassium, all concentrations of adrenaline less than 1 in 50,000 increase the excitability to A.C.; in some muscles adrenaline may not depress the excitability to A.C. even in concentrations of 1 in 10,000. In summer muscles even saturated solutions (in *Mytilus* saline) of veratrine or caffeine increased the excitability to A.C. It is remarkable that *Mytilus* muscle preserves its irritability in such high concentrations of these substances.

In summer muscles caffeine (saturated solution in *Mytilus* saline) has certain effects resembling those of calcium (1) in opposing the depressant action on the excitability to A.C. of adrenaline (1 in 50,000) and veratrine (saturated solution in *Mytilus* saline), (2) in producing a sudden drop in excitability, followed by a partial recovery, when it is withdrawn. The sudden drop in excitability to A.C. after withdrawal of caffeine implies an increased sensitivity to chemical stimulation which in sensitive muscles may culminate in a contraction when the drug is withdrawn. No such effect was observed on withdrawal of adrenaline or veratrine. In winter, the effects of caffeine and adrenaline or veratrine are synergistic.

Iodoacetic acid (1 in 7000) has a similar effect to that of anions and the drugs. Asphyxia, produced by bubbling nitrogen instead of oxygen through the muscle chamber, at first decreases the excitability to A.C. and increases that to potassium; subsequently excitability to both diminishes. Treatment with urea (5 p.c.) increases the excitability to potassium.

In performing these experiments the excitability of the muscle was at first brought into a steady state as regards the response to A.C. and to potassium. The muscle was then immersed in the experimental solution. This procedure was adopted as the muscle took a long time (sometimes a couple of hours or more) to recover from the effects of the substances, even though the time of exposure of the muscle to the action of drugs was as short as 10–15 min., an effect comparable to that of quarternary ammonium salts on frog muscle-nerve preparations [Ing & Wright, 1931].

Influence of initial length. The contrast between the A.C. and the potassium contractions was emphasized by a study of the effect of varying the initial length upon the tension produced. In these experiments the muscle was stretched slowly at the rate of about 1 mm. in 2 min. The length was at first increased in steps of 3–4 mm. and was then decreased in steps by a similar amount, the muscle being stimulated after each variation in length. In the first series of observations the muscle was stimulated with A.C.; in the next by potassium and, in some experiments, a third series of observations was recorded, in which the muscle was again stimulated with A.C. to see that there was no alteration in the optimum length during the course of the experiment. Usually the muscle was stretched by a length of 6–9 mm. in all; if the muscle was stretched more than this, it was difficult to perform experiments with diminishing length, because the muscle did not recover its original length. Hence, in some experiments, the tension produced on stimulation

rapidly diminished as the length was decreased, since the full tension could not be recorded, the muscle contracting in part isotonically and then isometrically.

The optimum initial length for maximum response to A.C. is more than that for the maximum response to potassium when examined in sea-water. In *Mytilus* saline this relation is reversed, the optimum length for the response to potassium being more than that for the response to

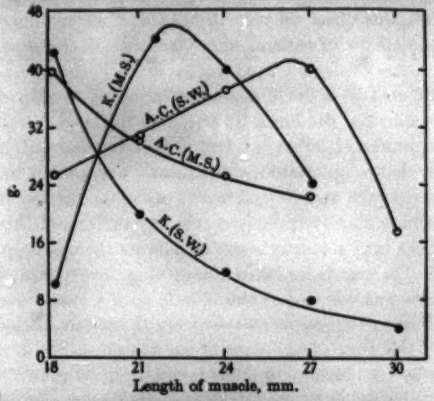

Fig. 7. Influence of initial length on tension produced by potassium (0·1*M*) and A.C. (10V.–10 sec.) in *Mytilus* saline (M.S.) in (sea-water (S.W.).

A.C. (Fig. 7). *Mytilus* saline differs from sea-water in two respects; firstly its *p*H is 7 instead of 8·4, secondly it contains no magnesium. In four experiments (one in winter and three in summer) alteration in the *p*H of *Mytilus* saline produced no alteration in the optimum length for A.C., but addition of magnesium (0·018*M* $MgCl_2$) altered the optimum length for A.C. in three muscles in summer, but had no effect in the one experiment in winter. It may be recalled that the effect of magnesium on the excitability to A.C. in winter differs from that in summer.

Effect of electrical stimulation. The excitability to A.C. and potassium is again varied in opposite directions by previous electrical stimulation. Repeated stimulation with A.C. first increases the excitability to A.C. (staircase phenomenon) and diminishes that to potassium. Subsequently the excitability to A.C. diminishes (fatigue) and that to potassium increases. Frog sartorius also becomes more sensitive to potassium during

fatigue [Gelhorn, 1932]. These effects on excitability, at first in one direction and then in the other, exactly resemble those of the anions or drugs, as if some such substance was liberated during A.C. stimulation.

A subthreshold stimulus, applied for a longer time, produces similar effects. Passage of 2 V. A.C. for 10 min. decreases, and passage for 1 hour increases the excitability to potassium.

During continued stimulation with A.C. for 5 or 10 min., after the initial contraction has declined, the excitability of the muscle to A.C. and to potassium is also affected in opposite directions; the threshold to A.C. rises and that to potassium falls.

Increased sensitivity to chemical stimulation during the passage also of a short A.C. stimulus is shown by the following experiment. The latent period of the barium contraction is sometimes very long (up to 1 hour). If such a muscle is immersed in *Mytilus* saline containing barium (0·02–0·07*M* $BaCl_2$) the muscle lies quiescent. Passage of A.C. (10 V.) even for 2 or 3 sec. is sufficient to excite the muscle; the barium then produces its typical powerful contraction, the muscle continuing to contract after the cessation of the current. Ordinarily passage of A.C. (8–10 V.) for such a short period produces little or no contraction.

After continued stimulation for 5 or 10 min., it takes about 10–15 or more minutes for the excitabilities of the muscle to be restored to their original values. This increased excitability, following prolonged passage of current, is especially noticeable in the response to drugs such as adrenaline or acetylcholine. Towards the end of recovery from exposure to a prolonged passage of current, the muscle often passes through a stage in which the excitability to potassium is low and that to A.C. high (cf. supernormal phase, Adrian, 1921], so that if potassium is added at varying times after the cessation of the current, the response may be great, small or normal.

In contrast to the recovery of excitability to A.C. from the depressant effect of excess of potassium, calcium (0·01–0·02*M* $CaCl_2$) delays the recovery of excitability to A.C. from the depressant effect of exposure to prolonged passage of the current.

The persistence of increased sensitivity to chemical stimulation in a winter (potassium sensitive) muscle is sometimes found even after a short A.C. stimulus; corresponding decrease in excitability to A.C. in similar muscles after a short A.C. stimulus is shown by the rapid onset of fatigue.

A similar mechanism is indicated in the phenomenon of a secondary spontaneous contraction which, in certain circumstances, follows the

response to a short A.C. stimulus (Fig. 8). This is apt to occur when the sensitivity of the muscle is such that the exclusion of calcium, or addition of excess of ammonium (0·282*M*) or potassium (0·1–0·2*M*) or sodium, just fails to produce a contraction (in the case of potassium this can be done by increasing the calcium content of *Mytilus* saline to twice that of sea-water). The secondary contraction resembles a potassium contraction but not a response to A.C. in the following ways: (1) it occurs in the absence of calcium; (2) when it occurs in *Mytilus* saline or in sea-water, it is inhibited by adding more calcium, or by previous stimulation with A.C.; (3) the optimum concentration of magnesium required is the same.

Fig. 8. Secondary contracture after an A.C. stimulus (8 V.–10 sec.).

Potassium is a more powerful stimulant than sodium; the contracture which is produced when the muscle is immersed in an isotonic solution of sodium chloride or sometimes in *Mytilus* saline is diminished or abolished if the sodium is replaced by lithium, and increased if part of the sodium (0·05–0·1*M* KCl) is replaced by potassium, so that sodium is a more powerful stimulant than lithium. No contraction has been observed after direct immersion of the muscle in ammonium rich solutions, but very few experiments have been performed to test the stimulating power of ammonium; six summer muscles were used which are usually insensitive to chemical stimulation. The secondary contracture, after a short A.C. stimulus described above, if produced in *Mytilus* saline, is abolished if the sodium of the *Mytilus* saline is replaced by lithium. If *Mytilus* saline is ineffective the secondary contracture is produced if part of the sodium (0·1–0·3*M*) is replaced with ammonium; and if both sodium and ammonium are ineffective, it may be produced if part of the sodium (0·05–0·1*M*) is replaced with potassium. The efficacy of these cations in producing the contracture therefore varies in the order $Li < Na < NH_4 < K$. As this contracture is probably due to increased sensitivity of the muscle to these cations after an A.C. stimulus, the stimulating power of the monovalent cations probably varies in the same order. The efficacy of these cations in producing contraction of muscle of some other marine animals varies in the same order [Lillie, 1909]; the monovalent cations cause *Mytilus* muscle to swell and gain base in the same order [Singh, 1938].

This secondary contracture is presumably analogous to the veratrine contracture of skeletal muscle, that is, spontaneous excitation occurring after cessation of the electrical stimulus. In *Mytilus* saline this secondary

contraction in *Mytilus* muscle may be attributed to the exciting action of sodium, for reasons given in connexion with the effects of withdrawal of calcium described above. A contracture resembling a veratrine contracture more closely, because sustained for a longer time, can be produced in *Mytilus* muscle by stimulating it in *Mytilus* saline or sea-water to which sodium chloride has been added. Similarly other contracture-producing substances, such as caffeine and sodium bromide, may excite after stimulation with A.C. Contracture-producing substances have a similar effect in skeletal muscle [Gasser, 1930].

Properties of the sodium contraction

When placed in isotonic solution of sodium chloride, most muscles contract strongly. When calcium or potassium, in concentration up to

Fig. 9. Neutralization of sodium contracture by A.C. (10 V.–10 sec.). Muscle immersed in Ca-free saline.

twice that in sea-water are added, they relax again. The muscles which do not contract when potassium and calcium are both excluded are those which are insensitive to potassium. Less insensitive muscles respond to withdrawal of both calcium and potassium, but not to that of one ion only. Muscles more sensitive to potassium (winter) respond to the withdrawal of calcium alone, while muscles, still more sensitive, respond to withdrawal of potassium alone. For reasons stated above, these contractions due to withdrawal of calcium and potassium are attributed to the exciting action of unantagonized sodium ions, the antagonistic action

of calcium against sodium being greater than that of potassium. If the sensitivity of the muscle is very high, then both calcium and potassium are unable to antagonize the action of sodium and the muscle exhibits increase in normal tone. Comparable contractions of other smooth muscle have been described by Clark [1921] in mammalian muscle, and by Thornton & Gillespie [1937] in bronchial muscle.

The sodium contraction produced by withdrawal of calcium can be inhibited by stimulation with A.C. as it can be by treatment with potassium or by restoration of the calcium (Fig. 9). But this may be ineffective if the muscle is very sensitive to potassium as in winter, or if a less sensitive muscle is washed with a calcium-free saline for a long time (about 2 hours). Adrenaline (1 in 100,000) also inhibits the sodium contracture.

Properties of the barium contraction

The sensitivity of the muscle to barium is increased by those substances which increase the sensitivity to potassium. The optimum length of the muscle is the same for the two substances, as are the seasonal variations in excitability. Potassium increases the latent period (sometimes up to an hour) of the barium contraction, as it does with the sodium contraction.

Properties of the contraction produced by change of tension

Unstriated muscle has been known to contract on sudden stretch [Straub, 1900; Bayliss, 1902; Grützner, 1904]. During experiments on viscosity of *Mytilus* muscle by the method of isotonic stretch and release from a stretch [Winton, 1930], curves were obtained of such a form as would be produced if the muscle contracts when it is released from a stretch. Contraction of the muscle on stretch was very frequent. In *Mytilus* saline such a response to stretch does not usually occur in summer muscles, and not invariably in winter muscles. If muscles are placed in sea-water immediately after dissection and kept in sea-water thereafter, the response to stretch is absent, as mentioned by Winton [1937], but if the muscles are immersed for a time in one of a variety of solutions specified below, they become sensitive to stretch and remain so, even after putting them back into sea-water. The inhibitory action of sea-water is due to its large magnesium content.

I have obtained contraction on stretch in over sixty muscles, and I have not come across a single muscle in which, by suitable treatment, a contraction on stretch could not be elicited. Fig. 10 shows a record from

a muscle which contracted on stretch the first time but not the second. Fig. 11 shows a record for a muscle, the length of which was suddenly increased and decreased; the muscle contracted each time the length is varied. The curve produced on release is not merely due to viscous elastic recoil of the muscle; it will be seen that there is a definite latent period between the release and the contraction, the intermediate period being occupied by a curve produced by the viscous-elastic properties of the muscle. This latent period can be increased by potassium. In pure sodium chloride the contraction begins immediately after stretch or release; 0·01*M* KCl increases the latent period to 5–60 sec. and 0·02*M* KCl to 40–100 sec.

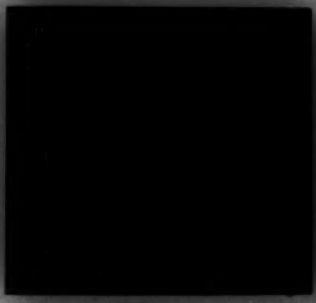

Fig. 10.

Fig. 11.

Fig. 10. Contraction of muscle on stretch with 10 g. weight. The muscle contracts the first time and not the second. Stretched at arrows ↓ and released at ↑.

Fig. 11. Effect of stretch and release in Ca-free saline containing 0·02*M* KCl. The muscle contracts each time it is stretched (X) and each time it is released (Y).

The response to stretch or release resembles that to potassium or sodium in the following ways: (1) it is inhibited by calcium, possibly due to an increased accommodation rate, (2) it is augmented during calcium deficiency in hypotonic solutions, and in solutions of anions and drugs such as adrenaline, veratrine, caffeine and nicotine that increase the excitability to potassium, (3) the optimum *p*H is 7·8, and (4) the contractions produced by change of tension are, like the sodium and potassium contractions, characterized by slow relaxation. Summer muscles (inexcitable to potassium) failed to respond to change of tension; winter muscles (hyperexcitable to potassium) were very sensitive to change of tension. Muscles which are initially insensitive to mechanical stimulation

can be rendered sensitive by immersion in a solution of any of the substances mentioned above (Section 3) as augmenting the response. Immersion in *Mytilus* saline, buffered at *p*H 7·8 with *M*/300 borate, is a reliable way of inducing sensitivity to stretch. In hypotonic solution (0·6 p.c.) of sodium chloride, stretch with as little as 2 g. evokes a response. In hypotonic solutions of calcium or magnesium chlorides, the stretch response is absent.

The muscle recovers its excitability to stretch when replaced in *Mytilus* saline after having been immersed in 0·56*M* KCl with 0·01*M* $CaCl_2$ for about 3–4 hours or more, if the *p*H of the solution is kept at 5–4·4. Such high concentrations of potassium do not cause irreversible changes in excitability even if calcium is excluded.

The contraction is not due to injury, as that is unlikely when the tension is increased. It is probably not due to stimulation of nervous elements, because drugs such as novocaine and nicotine (1 p.c.) increase rather than decrease the sensitivity to stretch.

When the muscle is placed in a solution of any substance, except potassium, present in concentration insufficient to cause a contraction, stretch or release evokes a contraction, which is more or less characteristic of the contraction usually produced by the substance in solution. Thus, in the absence of calcium, the contraction is characterized by a slow rate of relaxation like that of the sodium contracture. In excess of calcium (0·07*M*) or barium, the magnitude of the response is least in calcium and greatest in barium, corresponding with the stimulating power of these ions. Similarly among the anions, the stimulating power as well as the sensitizing effect to stretch varies in the same order. In the solutions of the drugs the rate of relaxation is slow in alkaline solutions (*p*H 7·8) and rapid in acid solutions (*p*H 7). If these substances have already made the muscle contract, then the response to stretch is feeble or absent.

It thus appears that change of tension sensitizes the muscle to these substances or lowers the threshold to stimulation by them. This is well seen in solutions of barium (0·01*M*). During the long latent period preceding the response to barium, the muscle is exceedingly sensitive to mechanical stimulation. Even a trace of barium sensitizes the muscle to stretch.

That the response to mechanical stimulation is to be regarded as due to excitation by sodium, rather than by potassium, when the muscle is immersed in *Mytilus* saline or sea-water is shown by (1) its ready occurrence in potassium-free solutions, and its inhibition by potassium even in those small concentrations which promote the response to A.C., (2) its

disappearance if sodium chloride is replaced by glucose or lithium chloride, though the latter sensitizes the muscle to potassium.

Tonus in isolated muscle

The contractions of *Mytilus* muscle can be placed into two groups, those having the properties of the response to A.C. and those having the properties of the response to potassium. Into which category should be placed the spontaneous contracture that occurs when a muscle is immersed in *Mytilus* saline or sea-water?

The tension which develops spontaneously in a muscle when immersed in *Mytilus* saline has properties resembling those of the potassium contraction, rather than those of the response to A.C., in the following respects: (1) tone is characterized by high viscosity [Winton, 1930]; (2) a muscle exhibits a high tone if its sensitivity to potassium is high and that to A.C. low; (3) calcium up to a concentration twice that of sea-water diminishes tone; (4) tone decreases with moderate decrease in *p*H; (5) in winter muscles, both the excitability to potassium and tone increased with decrease of temperature (experimental range, 4–20° C.); (6) low concentrations of adrenaline decrease the excitability to potassium and also inhibit tone.

The only ion that is present in *Mytilus* saline or sea-water in a stimulating concentration is sodium; calcium, potassium and magnesium are present in concentrations in which they inhibit tone. Consequently tone in isolated muscle is probably a sodium contracture. This view is supported by the following observations: (1) Sodium contracture is abolished during the period following an A.C. stimulus (Fig. 9). Spontaneous contracture in *Mytilus* saline is similarly abolished. No other contracture is abolished and redevelops in the same way. The high viscosity associated with sodium contracture and spontaneous tonus is reduced during the period following an A.C. stimulus, provided the muscle has not been deprived of calcium for long. (2) If the muscle exhibits excessive tone, it is reduced when the sodium is partly replaced (up to 30 or 40 p.c.) with glucose or lithium. Ordinary tone is not affected by similar treatment, probably owing to glucose possessing contracture-producing properties. (3) Adrenaline inhibits the sodium contracture, as well as tone, provided the muscle is not stimulated by adrenaline. (4) If a winter muscle is immersed for 16 hours in isotonic solutions of chlorides of lithium, sodium, ammonium, potassium and magnesium, it is found relaxed in all these solutions, except in that of sodium chloride; in this it is contracted to its maximum extent.

Antagonism between tone and twitch. When the muscle exhibits much tone, the excitability to A.C. is low. If the muscle exhibits a slight contracture, then the threshold voltage required to superimpose a small contraction (of about 2 g. tension) is greater than if the muscle is completely relaxed. Tone greatly increases if the muscle is immersed in *Mytilus* saline (buffered with *M*/300 borate at *p*H 7·8) for some hours, or if the animals are previously cooled [Singh, 1938]. Under these

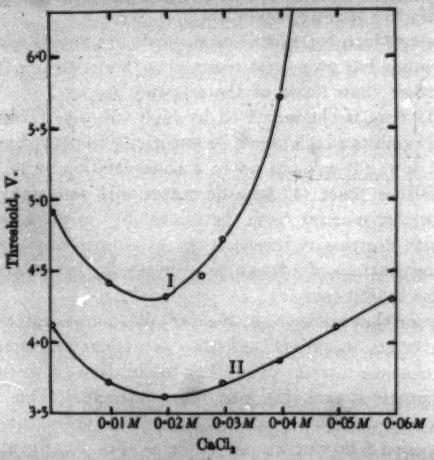

Fig. 12. Effect of calcium on excitability to A.C. Curve I was obtained with the muscle in slight contracture (3 g.). Treatment with excess of calcium had abolished this tone and the muscle was relaxed when Curve II was obtained. The voltage in Curve I was obtained after each increase in the concentration of Ca, and that of Curve II after each decrease in the concentration of Ca.

conditions the excitability to A.C. is low; in fact, the muscle may be inexcitable to A.C. (10 V.–10 sec.). The depressant effect of the excess of cations is greater if the muscle exhibits much tone (Fig. 12). The depressant effect of the monovalent cations and anions on the excitability to A.C., and their augmenting effect on the excitability to potassium, varies in the same order as their stimulating power; the stimulating power and the depressant effect of calcium is less than that of potassium. Seasonal variations in excitability to A.C. and potassium are also affected in opposite directions. We may conclude that the ionic environment

favourable to the response to stimulation with A.C. is antagonistic to the contracture produced by chemical stimulation. The antagonism between A.C. and contracture is further shown by observations: (1) that concentrations of potassium or calcium (in excess of 0·02*M*) which depress the excitability to A.C. cause the muscle to contract and convert an A.C. twitch into a contracture, the rate of relaxation being slow; (2) withdrawal of calcium or potassium depresses the excitability to A.C. and causes the muscle to contract; the A.C. twitch is converted into a contracture (Fig. 13); (3) as a result of a short A.C. stimulus (10 V.–10 sec.)

Fig. 13. Effect of Ca and NaCl on A.C. contraction. The muscle was stimulated at each arrow with A.C. (10 V.–10 sec.). First contraction in *Mytilus* saline; second contraction after immersion for 15 min. in Ca-free saline; third contraction in *Mytilus* saline again; fourth contraction in *Mytilus* saline to which NaCl was added (total NaCl 1·8 times normal).

the sodium contracture or tone may be neutralized (Fig. 9); (4) as a result of passage of A.C. for 5–10 min., the sodium contracture or tone is abolished, the muscle relaxes and contracts again on the cessation of the current; (5) in summer moderate excess of calcium, ammonium or potassium did not produce a contraction or convert an A.C. twitch into a contracture, and also did not depress the excitability to A.C.

The fact that unstriated muscle exhibits spontaneous tone, whereas striated muscle does not, may be regarded as a further instance of the rule enunciated above concerning the mutual antagonism between sets of conditions, which favour the response to A.C. and that to chemical stimulation, for unstriated muscle is more readily stimulated by chemical agents, whereas striated muscle is more readily excited by an electrical stimulus. Corresponding to this the depressant action of various substances on the excitability to electrical stimulation is greater in unstriated muscle than in striated muscle, the properties of *Mytilus* muscle resembling those of striated muscle more in summer than in winter.

Inexcitability in freshly dissected muscle. An interesting question in connexion with the action of sodium-deficient solutions is the excitability

of a freshly dissected muscle to A.C. Dulière & Horton [1929] suggested that the inexcitability of freshly dissected frog muscle was due to the escape of potassium into the interstitial spaces. Frog muscle contains potassium as its main ionic constituent, but *Mytilus* muscle contains sodium as its main ionic constituent. In certain circumstances,

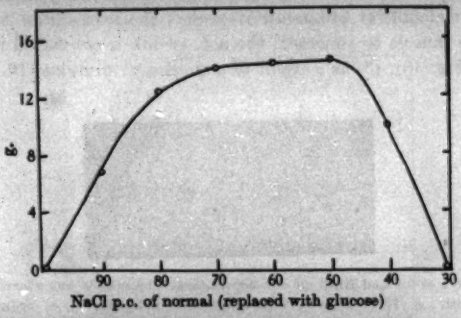

Fig. 14. Restoration of excitability to A.C. (10 V.–10 sec.) by treatment with a sodium-deficient solution (replacement of sodium chloride of *Mytilus* saline with equivalent amount of glucose).

Mytilus muscle is also inexcitable after dissection, particularly in winter, and in muscles taken from animals which have been kept at too low a temperature or for too long a time in a refrigerator. It may be significant in this connexion that it is just this type of muscle which has been found to contain an abnormally high sodium content [Singh, 1938].

Inexcitability in such muscles is in some way due to the action of sodium, as the excitability can be restored by treating the muscle with a sodium-deficient solution, such as hypotonic *Mytilus* saline, with an osmotic pressure of about 60 p.c. of normal, or by replacing 30–40 p.c. of sodium chloride by an osmotically equivalent amount of glucose (Fig. 14). Excitability can sometimes be restored by treatment of the muscle with an isotonic solution of potassium chloride for a few seconds, with adrenaline (1 in 100,000), or sometimes with lithium saline. This again supports the suggestion that the inexcitability is due to the action of sodium, as potassium and adrenaline antagonize the action of sodium, and the stimulating power of lithium is less than that of sodium or potassium, as is its corresponding power in diminishing excitability to A.C. In muscles that had been stored in the refrigerator for a very long

time (3–4 weeks), the excitability could not be restored by treating with a sodium-deficient solution, but was restored by an aerated phosphate buffered *Mytilus* saline at a *p*H of 7 to 6·5.

The phase of increased excitability in these muscles, following immersion in a sodium-deficient solution or in lithium chloride, is followed by a phase of depressed excitability, unless the original sodium content is restored. The increase of excitability following immersion in a sodium deficient solution is greater if the osmotic pressure is kept normal.

This inexcitability is probably due to the tonic contraction of the muscle and consequent increase in viscosity, produced as a result of dissection. Handling plays a part, as the inexcitability could sometimes be obviated by taking care, during dissection, not to touch the muscle with the fingers or the blade of the knife, or by dissecting it under sea-water or *Mytilus* saline, otherwise the muscle is made to contract by contact stimulation. This is in some way related to the fact that substances which produce swelling of the muscle cause it to become inexcitable, and mechanical contact, such as blotting with filter paper, increases the swelling [Singh, 1938].

Discussion

The two contractions due to stimulation by A.C. and potassium respectively, appear to represent two systems in *Mytilus* unstriated muscle which are probably the same as the phasic and postural contractile mechanisms in the dog retractor penis [Winton, 1930]. The A.C. contraction is characterized by a rapid rate of relaxation, probably due to low viscosity; the sodium and potassium contractures are characterized by a slow rate of relaxation probably due to high viscosity. The A.C. contraction, therefore, represents a system for the production of quick sharp contractions of low viscosity [Winton, 1937]; the potassium contraction represents a system for the production of tonic contractions, the purpose of which is to maintain a given tension or length. The ions favour ciliary movement in the same order as they favour the A.C. contraction; excitation resulting in ciliary movement is therefore probably produced by the phasic system. This is understandable, as ciliary movement will be facilitated by a low viscosity. The antagonism between the two systems is also understandable; rapid movement will be hindered by a high viscosity; so also a tonic contraction will be difficult to maintain if the viscosity is low.

It is known that if the potassium content of frog sartorius diminishes, the excitability to electrical stimulation decreases [Fenn & Cobb, 1934].

Irritability of *Maia* nerve decreases with potassium leakage [Cowan, 1934]. If *Mytilus* muscle is immersed for some hours in *Mytilus* saline, the potassium content diminishes [Singh, 1938] and the excitability to A.C. decreases. This loss of excitability may be restored by treating the muscle with a potassium-rich solution and then replacing it in *Mytilus* saline. Again, if *Mytilus* muscle is placed in excess of potassium or ammonium ions, the excitability to A.C. increases after a preliminary diminution. The initial decrease in excitability on addition of potassium or ammonium is probably determined by the presence of these cations outside the fibres, and the subsequent increase in excitability is probably determined by these cations inside the fibres. This view is supported by the fact that the recovery in excitability is rapid with ammonium and potassium, which are known to penetrate cells rapidly, and is much slower with sodium, which is known to penetrate much more slowly. Ing & Wright [1931] came to a similar conclusion on the comparison of the effects of the quarternary ammonium salts with those of ammonium chloride on frog muscle nerve. As with *Mytilus* muscle, ammonium salts produce a depression in excitability followed by a recovery. The recovery is more rapid with ammonium salts, which penetrate rapidly, than with quarternary ammonium salts which penetrate much more slowly. It is significant to note that *Mytilus* muscle does not recover from the depressant action of anions to which frog muscle is known to be very little permeable.

Conditions which result in reduction of total base of the muscle, for example, by placing the muscle in a hypotonic solution, make the muscle less excitable to A.C. Conditions which result in increase in total base of the muscle, such as by placing it in a hypertonic solution, increase the excitability to A.C. Cowan [1934] found that the injury potential of crab nerve was increased by placing the nerve in a hypertonic solution. Moreover, the order in which the monovalent cations affect the sensitivity to A.C. and ciliary movement ($Li < Na < NH_4 < K$) is the same as that found for their apparent ionic mobilities in protoplasm by Osterhout [1931]. Thus, as far as the ions are concerned, the sensitivity of the muscle to A.C. depends upon at least two factors: (1) the total ionic content of the muscle, (2) the kind of ion and its apparent mobility in protoplasm.

The main factor, therefore, responsible for the production of the A.C. contraction, is probably the potassium inside the muscle fibres, and may be termed the potassium system; excess of potassium outside the fibres causing tonic contraction and loss of excitability to A.C. as described

above. The tonic contraction in *Mytilus* saline or blood is probably produced by the sodium of the muscle or the medium [Singh, 1938], and may be termed the sodium system. Calcium or an A.C. stimulus neutralizes a sodium contracture, but if the muscle is deprived of calcium for long, then the A.C. stimulus fails to neutralize a sodium contracture; the calcium has probably diffused away. Many workers are agreed that calcium is liberated during activity [for discussion see Fenn, 1936]. In the absence of calcium the rate of relaxation of an A.C. contraction is decreased; the A.C. contraction then resembles a D.C. contraction. So there is probably a third system—the calcium system, which is the antagonist of the sodium system and reduces tone and viscosity during a phasic contraction produced by the potassium system.

Summary

1. The excitability to alternating current (A.C.) and the sensitivity to potassium are affected in opposite directions by the agents studied, viz. cations, anions, drugs, seasonal variations and electrical stimulation.

2. Immersion in solutions, containing excess of monovalent cations, Na^+, NH_4^+, K^+, renders *Mytilus* muscle first less and later more sensitive to stimulation with A.C. The excitability to potassium is affected in the opposite direction. Ultimately in excess of these abnormal cations, the excitability to A.C. declines.

3. The physiological effects of certain monovalent cations and anions, namely (1) their stimulating power, (2) their desensitizing effect on the response to potassium and their initial desensitizing effect on the response to A.C., and (3) their subsequent sensitizing effect on the response to A.C. and their desensitizing effect on the response to potassium, varies in the same order as they cause swelling and gain of total base [Singh, 1938]: $Li < Na < NH_4 < K$; $Cl < Br < NO_3 < CNS < CN$; (4) the stimulating power and sensitizing effect on the response to A.C. of the divalent cations varies in the same order as they cause swelling and gain of total base: $Ca < Sr < Ba$.

4. Restoring the muscle to *Mytilus* saline, after a period of immersion in a solution containing excess of monovalent cations K and NH_4 (that is, withdrawal), results in a phase of hyperexcitability before return to normal. Withdrawal of sodium also at first produces a preliminary increase in excitability to A.C., which is followed by a decline. The stage of hyperexcitability on addition and withdrawal of excess of NH_4^+ and K^+ is absent in muscles whose excitability to A.C. is low and to potassium high. The depressant effect is partly counteracted by calcium.

5. The types of contraction of this muscle can be placed into two groups: (1) the A.C. group, (2) the potassium group. The latter includes the contraction produced by chemical stimulation, mechanical stimulation, certain contractures produced as a result of A.C. stimulation, and spontaneous contracture or tone.

I wish to thank Dr F. R. Winton for his help in this work.

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THE EFFECT OF ANTERIOR PITUITARY EXTRACTS ON THE LIVER FAT OF VARIOUS ANIMALS[1]

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In a previous investigation Best & Campbell [1936] studied the action of a ketogenic extract of the anterior pituitary gland which produced an intense infiltration of fat and a rapid increase in the size of the livers of fasting rats. Daily administration of this extract for a period of 3 days to forty-seven fasting white rats resulted in a fourfold increase in total liver fat over the corresponding value in the same number of control animals. The subsequent observations of MacKay & Barnes [1937] and of Fry [1937] have confirmed these results.

The present study is chiefly concerned with the quantitative differences in the response of various species to treatment with this extract. The increase in liver fat and in the ketonuria produced by the administration of the anterior pituitary extract has been investigated in rats, guinea-pigs, mice and chickens. The studies on chickens, which were obtained in collaboration with Dr Leslie Kilborn, will be published in another communication.

Methods

Both male and female animals have been studied, but in a single experiment only one sex was used. In nearly all instances the effect of fasting alone and of fasting together with the administration of anterior pituitary extract was observed. The fasting animals were given water *ad lib.*

The methods for the determination of urinary ketone bodies, liver fat (fatty acids plus unsaponifiable matter) and body fat were similar to those described previously [Best & Campbell]. The fat content of mouse liver was determined by combining four or five livers and proceeding as in the case of rat liver. In the experiment described in Table IV

[1] A preliminary report of this work was made at the Annual Meeting of the American Physiological Society, March 1936.

where it was necessary to determine the fats before hydrolysis and to determine the water, protein and non-protein nitrogen fractions of the livers, the following procedure was employed. The rat livers were excised and combined, fifteen in a group. They were then rapidly weighed, minced three times in a fine grinder and pulped in a mortar. Samples taken for dry-weight determinations were dried at 110° C. to constant weight. The values were the same as when the tissue was dried in a vacuum desiccator. A sample (30 g.) of liver pulp was extracted repeatedly with alcohol and ether. For the first extraction 10 vol. of absolute alcohol were added, lesser amounts being used in subsequent extractions. Between extractions the liver residue was ground. Five extractions with hot alcohol and four with warm ether were made. The combined alcohol and ether filtrates were evaporated *in vacuo* to dryness, transferred with petroleum ether to a 100 c.c. volumetric flask and made up to volume. After standing until the fine precipitate of alcohol-soluble, non-fatty solid had settled out, aliquots of the fat fraction were evaporated *in vacuo* in round-bottomed flasks to constant weight. The weight of the alcohol-soluble, non-fatty solid was obtained by removing the remaining fat solution, adding slightly acidified water to dissolve the precipitate and diluting to the mark. Aliquots were then evaporated to constant weight. The alcohol- and ether-insoluble residue consists mainly of protein, and its weight was determined after drying to constant weight in a vacuum desiccator.

The nitrogen contents of all these fractions were determined by the macro-Kjeldahl method. The alcohol-soluble, non-fatty solid contains many non-protein nitrogenous constituents but the non-protein nitrogen is better determined in trichloracetic acid filtrates. To about 25 g. of pulped liver 4·5 vol. of water were added and the mixture was well shaken for 10 min. 1 vol. of 25 p.c. trichloracetic acid was added, the mixture was shaken and filtered after 15 min. The value of non-protein nitrogen in the filtrate was taken in preference to the nitrogen of the alcohol-soluble, non-fatty solid fraction.

The preparation of the anterior pituitary extract was the same as that used in our previous work on rats. The dry powder, which is termed the "anterior pituitary preparation" and is subsequently referred to as A.P.P., is stable and solutions of desired concentration can be made up, clarified by centrifuging and used for injection. 1 g. of the preparation contains the active material derived from 100 g. of fresh beef anterior pituitary glands. There appears to be little variation in activity between lots but one large sample was kept as a standard to control the potency of others.

The experimental animals were of uniform stock supplied by the Connaught Laboratories Farm. The rats were of the Wistar strain. Some of the mice were of the Webster strain while others were of a strain obtained from the Rockefeller Institute. The guinea-pigs were of an inbred albino strain.

Experimental results

In agreement with the conclusions of Mottram [1909] we felt that the most satisfactory index of a change in liver fat was its relationship to the initial body weight and we therefore utilized this ratio in analysing our data on liver and kidney fat, liver and kidney weight, etc. This index was also used in determining the effect of the A.P.P. on ketonuria.

Effect on liver fat of rats

(A) *Single injection.*

The data in Table I illustrate the effect of a single injection of saline (series I, 30 rats) and of the solution obtained from 62·5 mg. of A.P.P. (series I*a*, 40 rats). The animals were fasted from the time of injection and

Table I. Female rats

Series I. Injected once with 3 c.c. saline.
Series I*a*. Injected once with 3 c.c. extract equivalent to 62·5 mg. of A.P.P. per 100 g.
Fasted during the period of the experiment.

Series	No. of rats	Initial weight g.	Time elapsed after injection hr.	Loss of weight g.	Liver weight per 100 g. initial weight g.	Total liver fat mg.	Liver fat per 100 g. initial weight mg.	Liver fat p.c.
I	6	253	0	—	3·21	339	134	4·17
	4	248	24	14	2·55	397	161	6·31
	5	235	48	25	2·41	333	141	5·85
	5	237	72	28	2·19	328	138	6·30
	5	247	96	39	2·35	412	167	7·11
	5	243	144	52	2·07	285	117	5·65
I*a*	8	239	24	7	3·51	1137	475	13·6
	8	217	48	18	2·91	584	270	9·27
	8	244	72	26	2·65	511	210	7·91
	8	242	96	31	2·77	507	210	7·59
	8	239	144	53	2·40	311	129	5·41

groups were sacrificed at intervals for the determination of liver fat. There were only slight changes in the amount of liver fat in the control (fasting) rats injected with saline. This has been observed frequently in our studies on rats. If the data are calculated on the basis of the percentage of fat in the liver, however, an apparent increase during fasting is noted. This is due to the fact that the liver loses weight rapidly in the first 24 hr. of starvation.

The A.P.P. *caused a 3½-fold increase in liver fat in the first 24 hr.* During the following 24 hr. a rapid decrease occurred. This decrease continued at a diminishing rate until at the end of 6 days the liver fat was again at the control level.

(B) *Repeated injections.*

In Table II the changes in the liver fat over a period of 4 days, when the injections of the A.P.P. were given daily to fasting rats, are described. Under these conditions a rise in liver fat occurred up to the 3rd day; the

TABLE II. Female rats

Series II. Injected once daily with 3 c.c. saline.
Series IIa. Injected once daily with 3 c.c. extract equivalent to 75 mg. of A.P.P. per 100 g. rat.
Fasted during the period of the experiment.

Series	No. of rats	Initial weight g.	Days of fast and injection	Loss in weight g.	Liver weight per 100 g. initial weight g.	Total liver fat mg.	Liver fat per 100 g. initial weight mg.	Liver fat p.c.
II	5	203	0	—	4·40	331	163	3·71
	5	207	1	16	2·81	303	146	5·21
	2	187	2	19	2·78	321	172	6·20
	2	219	3	31	2·47	277	127	5·14
	2	187	4	30	2·51	197	105	4·20
IIa	7	198	1	—	4·08	1047	529	12·9
	8	199	2	22	4·24	1162	584	13·7
	8	197	3	28	4·23	1224	620	14·6
	8	199	4	34	3·93	1105	555	14·1

increase being greatest on the 1st day and progressively less on the 2nd and 3rd days. On the 4th day the amount decreased despite the continued injection of extract. A similar fall has also been noted in guinea-pigs receiving daily injections of extract.

(C) *Three daily injections of various amounts.*

At one stage of these investigations we desired to develop a method for assaying the material from the anterior pituitary gland which produces an increase in liver fat. For this purpose we utilized the response of rats to injection of the material. As fasting female rats gave a maximum response after three subcutaneous injections at daily intervals, this procedure was used for the test. Fairly large groups were employed (usually twenty animals) to allow for individual variation. Since the effect of only one dosage was tested at a time, a group of twenty control animals was injected with 1 vol. of saline corresponding to that of the extract given. There is undoubtedly more variation in the results than would have

occurred if all the dosages had been tested at one time on one lot of animals.

The data in Table III show that under the conditions of this experiment the liver fat increases with the amount of A.P.P. administered.

TABLE III. Fasting female rats

Series *a*. Injected daily for 3 days with varying doses of A.P.P.
Series *b*. Injected daily for 3 days with the corresponding volumes of saline.

Daily dose of anterior pituitary preparation mg. per 100 g. rat	No. of animals		Difference in liver weight of *a* over *b* per 100 g. initial weight g.	Liver fat mg. per 100 g. initial weight		
	a	*b*		*a*	*b*	Increase
6·8	19	19	−0·19	145	114	31
14·9	20	20	+0·39	181	107	74
30·5	18	16	+0·64	249	120	129
60·6	20	20	+1·26	444	113	331
96·8	19	20	+1·58	629	160	469

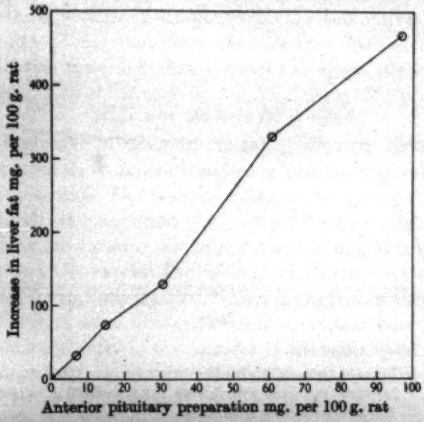

Fig. 1. Fasting female rats. Series *a*: injected daily for 3 days with A.P.P. Series *b*: injected daily for 3 days with the corresponding volumes of saline. The increases in liver fat (ordinates) refer to the increases of *a* over *b*.

Analysis of the data indicates that the dose-response curve (Fig. 1) is best treated as a straight line of slope,

$$\frac{d\,(\text{increase in liver fat, mg. 100 g. rat})}{d\,(\text{dose, mg. A.P.P. per 100 g. rat})} = 4{\cdot}5.$$

The statistical treatment of the results will not be discussed here as it soon became apparent that the test could only be used when doses of at

least 30 mg. of A.P.P. per 100 g. rat were given daily for 3 days. This dosage can only be attained when large amounts of concentrated extract are available.

Effect on the liver fat of guinea-pigs

The effect of the A.P.P. on the liver fat of fasting female guinea-pigs was determined in experiments somewhat similar to those carried out on rats. Groups of female guinea-pigs were injected daily with A.P.P. while the control animals received at the same time equal volumes of saline.

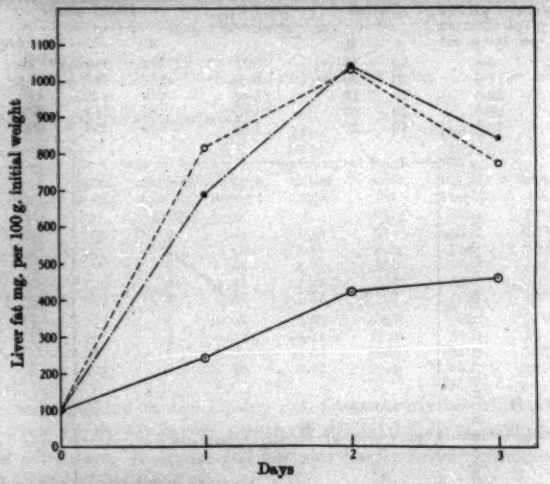

Fig. 2. Fasting female guinea-pigs.

○— — —○ Injected once daily with 10·0 mg. A.P.P. per 100 g. initial weight.
•——·——• Injected once daily with 3·8 mg. A.P.P. per 100 g. initial weight.
⊙———⊙ Injected once daily with saline.

The liver fat in a group of fed untreated animals was determined to secure the initial value. At appropriate intervals, up to the end of 3 days, the liver fat in groups of control and test animals was determined. The data obtained in this species are summarized in Fig. 2. They show that an increase in liver fat occurs in fasting guinea-pigs receiving daily injections of the A.P.P. The curves are similar in general form to those obtained in female rats under similar conditions (Table II). Thus we observe a great increase in the 24 hr. following the first injection and a lesser rise on the

2nd day. On the 3rd day despite the continued injection of extract the liver fat decreases.

There are, however, notable differences between the responses of these two species to the extract. (1) The dose of extract required to produce a given increase in liver fat is much less in guinea-pigs than in rats. (2) There is a steady increase in the liver fat of fasting guinea-pigs during 3 days of fasting. Expressing liver fat as the percentage of fat in the tissue the initial level was 3·0 which rose on successive days of fasting to 8·1, 12·3 and 14·8. As was pointed out above, no definite increase in the amount of liver fat occurs in fasting rats under our experimental conditions.

It is, therefore, apparent that guinea-pigs exhibit a greater increase in liver fat due to (*a*) fasting, and (*b*) administration of anterior pituitary extracts than rats under similar conditions. The former species might therefore be used for assay of the potency of the A.P.P. If such a step were contemplated, it would appear that the liver fat should be determined 24 hr. after the injection of a sample. While the level of liver fat is greatest on the 2nd day of injection the control (fasting) liver fat also increases markedly in this time and thus reduces the effect attributable to the extract alone.

Effect on the liver fat of mice

At the beginning of the work on mice, when the anterior pituitary extract was given daily, large variations in liver fat were encountered and some of the mice died. This was surprising at the time as the extract had never produced any apparent toxic effects in rats or guinea-pigs. It developed that the variable results and frequent deaths in mice were due to the fact that the extract had been given in too large a dose or was allowed to act for too long a period. This is illustrated by the curves shown in Fig. 3. The data were obtained from three experiments carried out at different times and refer to the increase in liver fat due to fasting alone and to fasting after a single injection of 10 mg. of A.P.P. From other data [Campbell] it was known that this dose was approximately maximal for the mouse. The liver fat was determined in groups of four mice.

The lower curve shows that fasting causes a considerable increase in liver fat in 24 hr. Within this period the values show a fairly uniform rise. After this time, however, large fluctuations are encountered and the liver fat may rise to very high or sink to very low levels. This behaviour seemed to be related to the amount of depot fat, for it was evident that in animals which had good stores, fatty livers were present and in those in which the fat reserves were depleted, the liver fat was low. Values illustrating this particularly well are those obtained from two groups of mice fasted for

7 days. An active, healthy group of mice showed, at this time, ample stores of body fat and high liver fat, the values of which were 14·5 g. and 1500 mg. per 100 g. of body weight respectively. At the same time a group of weak mice with low body temperatures had 3·9 g. of body fat and 175 mg. of liver fat per 100 g. of body weight. The high value of 1500 mg. was equivalent to 28 p.c. of fat in the liver. Good stores of body fat apparently in many cases are associated with a high level of liver fat under these experimental conditions.

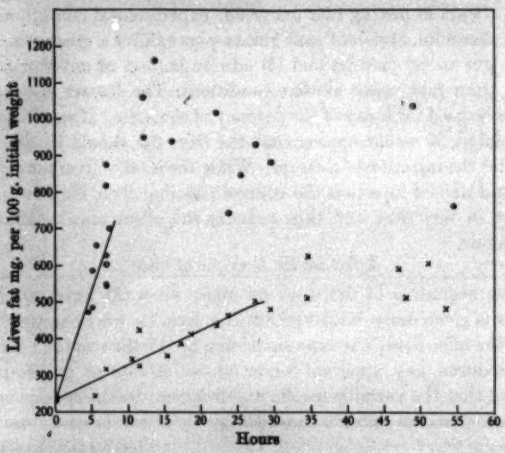

Fig. 3. Fasting female mice.

×—× Injected at 0 hr. with 0·4 c.c. saline.

⊙—⊙ Injected at 0 hr. with 40·0 mg. A.P.P. per 100 g. initial weight.

In mice which were fasted after a single injection of 10 mg. of A.P.P. a very sharp increase in liver fat occurred which was much greater than that due to fasting alone. The values showed more variation in this series than during fasting alone and in a shorter time, i.e. in 10 hr., became even more variable than the fasting values. The fluctuations in this curve seem to be dependent not only on the stores of body fat but also on the "sensitivity" of the particular group or groups to the active substance. It was noted that after long periods of fasting certain groups of mice with good stores of depot fat showed as large amounts of liver fat as were obtained

(within much shorter periods) in mice injected with the A.P.P. The response of mice to the material in anterior pituitary extracts causing an increase in liver fat will be discussed more fully in a later paper [Campbell].

Change in liver weight due to fasting

In mice, 7 hr. after the beginning of fasting the liver weight dropped to about 67 p.c. of the initial value. After this abrupt fall the liver weight *increased* until after about 15 hr. of fasting it reached the initial or a slightly higher level. This new level seemed to be maintained up to 3 days of fasting. As is shown in Fig. 3, the liver fat increased progressively from the beginning of the fast. Due to the dispersion of the values it was not possible to find, in mice, any quantitative relationship between the secondary rise in liver weight and the rise in liver fat.

In guinea-pigs during the first 24 hr. of fasting the liver weight fell rapidly. Thereafter, the liver weight increased slightly up to the completion of the 3 days of fasting which ended the period of observation. During the fast the liver fat increased steadily (Fig. 2) and the ratio

$$\frac{\text{increase in liver weight}}{\text{increase in liver fat}}$$

in the period of 24–48 hr. was 3·5 and in the period 48–72 hr. was 4·1.

In fasting rats the liver weight fell rapidly in the first 24 hr. but, in contrast to the findings in mice and guinea-pigs, after the initial large drop the liver weight continued to decrease. The liver fat remained constant or decreased slightly (Tables I and II).

Changes in liver weight due to injection of A.P.P.

The administration of the A.P.P. to fasting mice produced increases in liver weight far in excess of the control (fasting) values. Due again to the variable results, it is not easy to determine any quantitative relationships, if they exist, between the increase in liver fat and the increase in liver weight.

In fasting guinea-pigs also the administration of A.P.P. produced increases in liver weight above the control (fasting) levels. With the daily dose of 3·8 mg. of A.P.P. per 100 g. pig the ratio $\frac{\text{increase in liver weight}}{\text{increase in liver fat}}$ in 24, 48 and 72 hr. was 2·6, 1·6 and 2·1 respectively; and with the daily dose of 10 mg. the ratio at the same times was 2·7, 2·1 and 2·6 respectively.

The administration of A.P.P. to fasting rats also produced increases in liver weight above the control (fasting) levels (Table I). These animals received a single injection of A.P.P. at the beginning of the fast. Similarly,

the changes in liver weight of the animals which received daily injections of A.P.P. during fasting are given in Table II. In Table III the effect of varying doses of A.P.P. on liver weight are shown.

Comparison of the results of the three experiments shows that in fasting rats the injections of A.P.P. cause very similar changes in both the liver fat and liver weight. Close inspection of the data in Table I shows, however, that after the 4th day the liver fat decreases more rapidly than liver weight, and this is shown by the increasing values for the ratio $\frac{\text{increase in liver weight}}{\text{increase in liver fat}}$ which are 3·1, 4·0, 6·3, 9·6 and 7·7 on the 1st, 2nd, 3rd, 4th and 6th days respectively. In the experiment in which the fasting rats received daily injections of anterior pituitary extract the ratio of the increases is more uniform. It was 3·3, 3·5, 3·5 and 3·2 on the successive days.

The data of Table III refer to the experiments in which various daily doses of A.P.P. were given for 3 days to fasting rats. When curves are drawn for the increase in liver fat and the increase in liver weight against dosage, the points seem to fit a straight line in both cases. From this experiment the ratio has the average value of 3·1. We find, therefore, that shortly after the administration of anterior pituitary extract to fasting rats, for every gram increase in fat (fatty acids plus unsaponifiable matter), the liver retains approximately 2·1 g. of other materials.

In order to determine the quantitative relationship of the increase in liver fat to the increase in liver water and non-fatty solid constituents, the liver fats were determined as such by weighing after extraction with alcohol and ether. In this experiment female rats were fasted for 24 hr. previous to and after injection. A group of fifteen was injected with 150 mg. each of A.P.P. and the fifteen control animals were given an equal volume of saline. 24 hr. after the injection the livers were removed and fractionated. The data show (Table IV*a*) that when the A.P.P. was administered the fat fraction of the liver increased to the greatest extent, amounting to 51·3 p.c. of the total increase in liver weight. The water content also increased to an amount corresponding to 43·8 p.c. of the total increase. The alcohol-insoluble fraction represents the material not soluble in 89 p.c. alcohol and in ether and consists chiefly of protein. This fraction increased only slightly, 4·85 p.c. of the total increase, and the non-fatty solids soluble in 89 p.c. alcohol and in ether increased by only 0·071 p.c. In this latter fraction would be found many non-protein nitrogenous constituents, and the carbohydrates would be accounted for in the alcohol-insoluble or the non-fatty, alcohol-soluble fractions.

TABLE IV*a*. Fasting female rats

Series *a*. Injected once with 3 c.c. extract equivalent to 83 mg. of A.P.P. per 100 g.
Series *b*. Injected once with 3 c.c. saline.
Composition of the livers 24 hr. after injection.

	Weights of fractions				
	Series *a*		Series *b*		
Fraction of the liver	g. per 100 g. initial weight	p.c. of liver	g. per 100 g. initial weight	p.c. of liver	p.c. of the increase in liver weight Liver weight increase = 0·845 g. per 100 g.
Non-fatty solids:					
Alcohol insoluble	0·7200	19·70	0·6790	24·40	4·85
Alcohol soluble	0·0076	0·21	0·0070	0·25	0·071
Water	2·260	61·80	1·890	67·95	43·80
Fat	0·654	17·90	0·221	7·95	51·3
Totals	3·642	99·6	2·797	100·5	100·0

TABLE IV*b*. Fasting female rats

Series *a*. Injected once with 3 c.c. extract equivalent to 83 mg. of A.P.P. per 100 g.
Series *b*. Injected once with 3 c.c. saline.
Composition of the livers 24 hr. after injection.

	Weight of nitrogen			
	Series *a*		Series *b*	
Fraction of the liver	mg. per 100 g. initial weight	mg. per 100 g. liver	mg. per 100 g. initial weight	mg. per 100 g. liver
Non-fatty solids:				
Alcohol insoluble	102·00	2790	97·90	3520
Non-protein nitrogen	6·56	179	6·03	216
Fat nitrogen	5·08	138	4·62	128
Totals	113·6		108·5	
Total nitrogen	113·5		105·4	

The distribution of nitrogen (Table IV*b*) showed that the extracts produced an increase in the total nitrogen of the liver which amounted to an increase of 5·5 p.c. over the control liver nitrogen. Due to the increase in fat and water there was a decrease in the percentage of total nitrogen in the liver. Similarly the non-protein nitrogen increased slightly in absolute amount but fell in terms of percentage of liver weight. It is evident that the increase in liver weight was due mainly to fat and water, only a small part being due to protein-like constituents. If the increase in nitrogen of the alcohol-insoluble fraction be expressed as protein by use of the factor 6·24 [Addis *et. al*, 1936], an increase in protein of 25 mg. per 100 g. initial body weight is indicated.

Effect on ketogenesis of rats and guinea-pigs

The data show (Table V) that in fasting rats receiving saline injections there is no perceptible increase in the excretion of ketones and no increase

TABLE V. Female rats

Series V. Injected once daily with 3 c.c. saline.
Series V*a*. Injected once daily with 3 c.c. extract equivalent to 94 mg. of A.P.P. per 100 g. initial weight.
Fasted during the period of the experiment.

Series	No. of rats	Initial weight g.	Total ketone body excretion mg. per 100 g. initial weight per day Days 1	2	3	Liver fat at end of 3 days per 100 g. initial weight mg.
V	17	159	0·70	0·63	1·13	219
V*a*	22	159	1·39	22·80	32·40	673

in liver fat. The daily injection of anterior pituitary extract produces a striking excretion of ketones on the 2nd and 3rd days, but little increase on the 1st day. The liver fat, however (Table II), rises sharply on the 1st day and continues to increase slightly on the 2nd and 3rd days. There is, therefore, a decided lag in the production of ketone bodies as compared with the rise in liver fat in this experiment.

Since guinea-pigs respond more readily than rats with an increase in liver fat during fasting or after the administration of anterior pituitary extracts, it might be expected that they would also excrete more ketone bodies. This does not appear to be the case (Table VI). In these experiments the animals were fasted from the time of the first injection. The test animals received injections of A.P.P. daily for 3 days while the controls received saline. The ketone body excretion was followed from day to day, and at the end of 3 days the liver fat was determined. It can be seen

TABLE VI

Animals (fasting)	No. of animals	Daily injection of anterior pituitary preparation per 100 g. initial weight mg.	Total ketone body excretion in 3 days per 100 g. initial weight mg.	Liver fat per 100 g. initial weight mg.	Liver fat p.c.
Female rats	11	94·0	82·0	430	10·70
	22	Saline	2·46	138	5·50
Female guinea-pigs	5	4·6	7·44	647	17·10
	5	2·0	3·95	732	20·50
	5	Saline	2·68	382	12·80
Male guinea-pigs	5	4·6	2·33	316	9·20
	5	2·0	2·08	244	7·80
	5	Saline	1·86	157	5 62

(*a*) that in female guinea-pigs the liver fat is increased more readily than in males both by fasting and by fasting with the administration of anterior pituitary extracts; (*b*) the production of ketone bodies (due both to fasting and to fasting with the administration of anterior pituitary extracts) is greater in female guinea-pigs than in males; (*c*) female guinea-pigs do not excrete more ketones than female rats during fasting, although the increase in liver fat is much greater in the former. It can be seen also that when a sufficient dose of A.P.P. is given to rats to increase the liver fat by an amount similar to that produced by a smaller dose in guinea-pigs, the ketone body excretion is much greater in the rats.

There are, therefore, some large quantitative discrepancies between the accumulation of liver fat and the ketonuria under the influence of fasting and of anterior pituitary extracts. There are obviously several points which suggest a relationship between the two.

Histological observations on kidney fat and liver fat

Histological sections show that the livers of fasting rats, following injection of anterior pituitary extract, have a characteristic appearance due to the infiltration of fat. Using the hæmatoxylin-eosin stain, the fat is represented by vacuoles which are seen to be more numerous in the region of the portal veins than in the region of the central veins. The fat is distributed as fine globules scattered throughout the protoplasm of the cells and many examples can be picked out where the droplets form a ring around the nucleus. The appearance of this liver is different from a liver made fatty by diets poor in choline. In the latter case the fat droplets tend to coalesce and form large masses in the cell.

In co-operation with Dr D. L. MacLean a histological and chemical investigation was made of the effect of the A.P.P. on the weight and fat content of the kidney in relation to the corresponding changes in the liver. In this experiment, groups of fasting female guinea-pigs were injected daily for 2 days with 8·4 and 3·3 mg. of A.P.P. per 100 g. In addition to the controls injected with saline a group was injected with 8·4 mg. per 100 g. of brain extract prepared in the same way as the A.P.P. The chemical determinations (Table VII) showed that the brain extract produced a slight increase in weight and fat content of the liver over the control values. The changes were small in relation to those produced by the corresponding dose of A.P.P. The brain extract had no effect on kidney fat. The A.P.P. produced increases in kidney weight and kidney fat. The changes were proportional to the amount of A.P.P. injected, but were slight in comparison with the increases in liver weight and fat.

TABLE VII. Female guinea-pigs

Controls. Injected once daily with 2 c.c. saline.
Series *a*. Injected once daily with 8·4 mg. brain preparation.
Series *b*. Injected once daily with 3·3 mg. A.P.P.
Series *c*. Injected once daily with 8·4 mg. A.P.P.
These injections were calculated on the basis of mg. preparation per 100 g. initial body weight.
Fasted during the period of experiment (2 days).

Series	No. of animals	Initial weight g.	Weight loss g.	Liver weight per 100 g. initial weight g.	Liver fat per 100 g. initial weight mg.	Liver fat p.c.	Kidney weight per 100 g. initial weight mg.	Kidney fat per 100 g. initial weight mg.	Kidney fat p.c.
Controls	10	299	48	3·16	136	4·3	1·00	31·4	3·14
a	10	298	47	3·67	167	4·5	1·00	31·2	3·12
b	10	299	66	4·31	439	10·2	1·14	42·0	3·68
c	10	299	49	4·36	594	13·6	1·15	54·0	4·70

The histological observations, in general, supported the chemical findings, namely, that the brain extract produced a slight increase in liver fat and the A.P.P. a great increase in liver fat. The fat was distributed in fine droplets through the protoplasm of the liver cells. The anterior pituitary extracts also produced a fatty infiltration of the kidney, which was slight in relation to the fatty changes in the liver. The fat in the kidney varies in its distribution. It is usually seen in the loops of Henle and occasionally in the proximal convoluted tubules. In comparing the kidneys and livers of the individual animals, whether in the control (fasting) group or in the groups which received A.P.P., it was seen that where a particularly fatty liver was encountered, the kidney was correspondingly fatty.

It is apparent that anterior pituitary extracts can increase kidney weight and kidney fat and that these changes may be a reflexion, though much reduced, of the corresponding changes in the liver.

Effect on body fat

As in previous studies [Best & Campbell], we again found that the A.P.P. caused a fall in the body fat of rats. A group of fifteen female rats was fasted and injected daily for 3 days with 85 mg. of A.P.P. per 100 g. of body weight. The group of eight control animals was injected with an equal volume of saline (3·0 c.c.) at the same times. The carcass fat (body less liver) in the experimental group was 4·99 g. per 100 g. of body weight and in the control group was 5·65 g. The liver fats of these animals were 458 mg. per 100 g. of body weight and 131 mg. respectively. The decrease

in body fat due to the anterior pituitary injections was therefore 660 mg. and the increase in liver fat was 327 mg. About half of the fat lost from the depots may, therefore, be accounted for by that found in the liver under these conditions.[1]

The problem of the effect of the anterior pituitary injections on body fat was also attacked using fed guinea-pigs which received daily injections of extract during a 9-day period. The animals were fed a commercial ration and the daily consumption of food was measured. The ration given to the control animals (1 day later) was adjusted so that their food consumption was approximately equal to that of the experimental animals. 1 c.c. of extract, equivalent to 10 mg. of A.P.P., was given daily to the experimental animals, while the controls received 1 c.c. of saline.

After a period of 9 days the liver fat, intestinal fat and fat in the carcass was determined. The fat content of the intestines was found to make no appreciable difference in the results, so it has been included under depot fat (Table VIII). Two such experiments were done and in one of

TABLE VIII. Female guinea-pigs

Series *a*, *c*. Injected once daily with 1 c.c. saline for 9 days.
Series *b*, *d*. Injected once daily with 10 mg. A.P.P. per pig for 9 days.
Fed a mixed diet.
(The experiment of Series *a* and *b* was repeated with Series *c* and *d*.)

Series	No. of animals	Initial weight g.	Change in weight g.	Daily food intake g.	Body fat per 100 g. body minus liver g.	Liver fat per 100 g. initial weight mg.	Liver fat p.c.
a	20	287	3	13·5	6·00	146	4·08
b	20	268	−15	13·5	4·01	164	3·95
c	18	201	22	14·0	5·00	129	3·34
d	18	227	0	16·6	3·53	117	2·79

them (series *c* and *d*) the controls did not receive as much food as the animals to which the anterior pituitary injections were given. Despite this the body fat in the latter group fell below the control value. In series *a* and *b* there is a fall in body fat due to the anterior pituitary injections. The animals receiving extract also lost weight in relation to the controls.

It is noticeable that a daily dose of 10 mg. of A.P.P. for 9 days to fed guinea-pigs produced no increase in liver fat, but in fasting guinea-pigs receiving extract for 3 days a great increase in liver fat occurs (Fig. 2).

[1] The results of experiments with deuterium-containing fats made in co-operation with Dr Hugh Barrett and Miss Jessie Ridout indicate that all the liver fat comes from the body reserves under these conditions.

Discussion

The results of a single injection of the anterior pituitary extract in rats suggest that the intensity of action is definitely less at 48 hr. after administration than at 24 hr. This interpretation is not necessarily correct since it involves the assumption that the only action of the substance is to transfer fat from the depots to the liver. The rate of utilization of fat in the liver may have increased and this, rather than a decreased rate of mobilization from the depots, might cause a fall in the amount of fat in the liver. When the injections are repeated at daily intervals the findings suggest that the level of liver fat reaches its highest point on the 3rd day. In guinea-pigs the peak is attained somewhat earlier. These time relationships might be affected by such factors as dosage of the extract, the reserves of depot fat, etc. We cannot explain at present the falling off in liver fat while the injections are continued. This might be due to depletion of "easily mobilizable" fat, but there are still appreciable amounts of glycerides present in the depots when the decrease in liver fat occurs.

When three daily doses of A.P.P. were given to rats the increase in liver fat noted on the 3rd day was apparently directly proportional to the dose used (Table III, Fig. 1).

A comparison of the data in Table II with that in Fig. 2 shows that female guinea-pigs are much more susceptible to the A.P.P. than female rats. In guinea-pigs a daily dose of 3·8 mg. per 100 g. produced in 2 days an increase in liver fat above the initial level of approximately 940 mg. per 100 g. of initial body weight. In rats a dose of 75 mg. produced an increase of liver fat, calculated on the same basis, of 420 mg. The results on mice cannot be compared directly with those obtained in other species since the response in mice is much more rapid and, if observations are made after 2 or 3 days, the findings are extremely erratic. There is no doubt, however, that mice are as susceptible as guinea-pigs to the A.P.P. and because of their rapid response they are preferable for testing purposes.

In the present investigation the amount of fat in the liver of rats, which were fasted but not injected with A.P.P., tended to remain at the original level or to decrease. The ketone body excretion did not exhibit an appreciable increase during fasting. Other workers have found rats very resistant to ketogenesis [e.g. Levine & Smith, 1927], but in certain cases a fasting ketogenesis has been noted in this species [Cori & Cori, 1927]. The extent depended on the season at which the observations were made. Dible [1932] also observed an increase in the excretion of ketone

bodies in the fasting rat and a slight increase in liver fat. Mottram found that a great increase in liver fat occurred in fasting rabbits and guinea-pigs. This increase exhibited a periodicity which we have not observed. In our experience there is a steady and progressive increase in liver fat up to the end of 3 days' fasting in guinea-pigs. In mice kept at 0° C. for from 2 to 4 hr., Lánczos [1935] has reported an increase in liver fat during fasting. In the present investigation it is quite apparent that an increase in liver fat can occur in fasting mice to an even greater extent than that found in guinea-pigs. In mice or guinea-pigs in which the liver fat increases during fasting, after the initial definite fall in liver weight, an increase in weight occurs. In rats, on the other hand, after the initial fall the liver weight continues to decrease slowly. The values of the ratio $\frac{\text{increase in liver weight}}{\text{increase in liver fat (fatty acids)}}$ during fasting in guinea-pigs and in guinea-pigs and rats receiving A.P.P. are of the same order (average of 3·5 in fasting guinea-pigs, 2·6 in fasting guinea-pigs receiving A.P.P., and 3·2 in fasting rats receiving A.P.P.). These values are those observed 24 hr. after the beginning of fasting or after administration of A.P.P.

The increase in the liver weight of fasting rats receiving anterior pituitary extract for short periods is due chiefly to an influx of fat and water (Table IV*a*). In the livers of fasting rats 24 hr. after a single injection of extract, 51·3 p.c. of the increase in liver weight was due to fat, 43·8 p.c. to water, and only 4·9 p.c. to non-fatty solids. The protein nitrogen and non-protein nitrogen were increased slightly in amount by the injections, but due to the influx of fat and water the percentage of these values fell. It has been pointed out that if the injections of anterior pituitary extract are continued for longer than 3 days, or if the injections are stopped so that the liver fat decreases, the ratio $\frac{\text{increase in liver weight}}{\text{increase in liver fat}}$ will increase. Under these conditions it is indicated that the nitrogen content of the livers will increase out of proportion to the fat content. Effkemann & Herold [1935] found that in rats and pigeons anterior pituitary extracts and "unspecific organ extracts" produced increases in liver weight due to increases in nitrogen and water content and not to increases in fat or glycogen. Their experimental animals were, however, fed. It may be pointed out that in previous experiments [Best & Campbell], and in the experiments reported here, any increases in liver weight produced by extracts of the liver, pancreas or brain have not been nearly as great as those produced by similar doses of anterior pituitary extract. Schaffer & Lee [1935] observed that short periods (10–18 hr.) of treatment with a growth hormone preparation produced in fasting rats

a decrease in the percentage of non-protein nitrogen in the liver. We have confirmed this observation but found, as mentioned above, that the absolute amount of non-protein nitrogen in the liver showed a slight increase. This relation was caused by the increase in liver weight due chiefly to the accumulation of fat and water.

In the rather controversial subject of water storage in the liver with the various other constituents it is usually assumed that little or no water accompanies the deposition of fat. Kaplan & Chaikoff [1936] have presented strong evidence to show that in dogs under various experimental conditions liver water is directly proportional to a liver fraction the chief constituent of which is protein, and that water does not accompany liver fat or glycogen in appreciable amounts. They do not exclude the possibility, however, that water in small amounts may accompany liver fat or glycogen. The average value of the ratio of water to protein in dogs was found to be 4·3 by these workers. If the nitrogen content of the alcohol-insoluble liver fraction in rats be considered to represent protein (Table IV*a* and *b*), the ratio of water to protein was 3·09 in the controls and 3·55 after the injection of A.P.P. The proportion of water to protein, therefore, apparently increased slightly after the injection of the A.P.P. and it would appear also from the absolute increases in water and protein that there was not a strict relationship between them. It appears, however, that liver water is much more closely associated with protein than with fat.

The results of the present study on ketosis show, in confirmation and extension of the findings of Butts & Deuel [1933], that female animals excrete more ketone bodies than males. In Table V this difference may be observed in both the fasted guinea-pigs and in those which received A.P.P. This sex difference is also seen in the deposition of fat in the livers of mice and guinea-pigs to which A.P.P. has been administered. The results in the latter species are given in Table VI, while those in mice will appear in a subsequent publication by one of us (J. C.).

When the liver fat is increased in guinea-pigs by fasting or injection of A.P.P. there may be only an insignificant rise in ketonuria (Table VI). In rats, on the other hand, a smaller increase in liver fat after injection of A.P.P. is accompanied by a rather intense ketonuria. It will be remembered, however, that the amount of A.P.P. required to produce similar increases in liver fat is much greater in the rat than in the guinea-pig.

It would appear probable that the rise in liver fat precedes the increase in ketonuria. While the data in Tables II and V support this view the results in other experiments are by no means as definite. To make a

decision on this point large groups of animals would have to be studied at short intervals after treatment with A.P.P.

It may be pointed out that the effects of the A.P.P. are in some respects analogous to those of fasting alone. The evidence for this view has already been mentioned but may be summarized as follows. The increase in liver fat and ketogenesis produced by the A.P.P. are inhibited by feeding and are therefore best shown when the effects of the A.P.P. are superimposed on those of fasting. The increase in liver fat (due to fasting alone or to fasting with the administration of A.P.P.) is greater in mice and guinea-pigs than in rats. During fasting, female guinea-pigs respond more readily than males with an increase in liver fat and ketonuria, and this is also true of the responses to the A.P.P. The administration of A.P.P. produces a fall in body fat. It therefore appears that the mobilization of fat produced by fasting is intensified by the injection of A.P.P.

The significance of the slight but definite increase in kidney fat when the A.P.P. is administered is not clear. Further studies of this problem could profitably be made.

Summary

Some effects of an anterior pituitary preparation on the fat metabolism of rats, guinea-pigs and mice have been investigated. It has been shown that:

(1) In fasting animals the anterior pituitary preparation causes a much greater increase in liver fat in guinea-pigs and mice than in rats. Fasting alone produces a pronounced increase in liver fat in guinea-pigs and mice. This is greater in the latter species. The amount of fat in the liver of the rat is frequently decreased by short periods of fasting.

(2) Female guinea-pigs exhibit a greater increase in liver fat than males during or after the administration of the anterior pituitary preparation. The ketogenesis produced by the injections is also greater in females than in males (confirming Deuel). The ketogenesis produced in female guinea-pigs is not nearly as great as that produced in female rats when the liver fat rises to similar levels.

(3) The fat which accumulates in the liver of the fasting rat after anterior pituitary injections rapidly disappears on stopping the injections. Despite continued daily injections of the preparation to fasting rats or guinea-pigs, a decrease in liver fat occurs after several days.

(4) Associated with the increase in liver fat in guinea-pigs, due to administration of anterior pituitary preparation, there is an increase in kidney fat.

(5) In fasting rats or in fed guinea-pigs the preparation causes a fall in body fat.

(6) The preparation produces an increase in liver weight which is due chiefly to deposition of fat and water. A slight increase in non-fatty solid material also occurs.

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THE CENTRAL DEPRESSOR ACTION OF ADRENALINE AND ITS INHIBITION BY ERGOTOXINE

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THE rise in blood pressure produced by the injection of moderate doses of adrenaline in the rabbit is generally followed by a more or less pronounced fall, which may last for several minutes. Thus van Leersum [1911] observed in the unanæsthetized rabbit that the secondary fall, after a dose of $\frac{1}{2}$ mg. of adrenaline injected into an ear-vein, exceeded 30 mm. Hg and lasted for a considerable time. This phenomenon has been interpreted as being due to a reflex stimulation of the vasodilator centres [Trendelenburg, 1929] and it has been suggested that the effect is analogous to the vasodilatation and the slowing of the heart after injections of adrenaline in the dog, due to reflex actions from the carotid sinus and the aortic arch, as shown by Heymans [1929]. The accumulation of tissue metabolites, especially those of an acid character due to a diminished blood supply to certain areas of the organism, has also been considered as a possible cause of the secondary fall. McGuigan & Mostroem [1912] found that the effect was increased after infusion of acid into the circulation and diminished after infusion of alkali. A great number of observations concerning the vasodilator or depressor effects of adrenaline, in different animals and under different conditions, have been reported, but only those dealing with a central action will be considered here.

Observations on rabbits showed that ergotoxine or ergotamine could abolish the normal secondary lowering of the blood pressure, even when given in doses too small to affect the pressor response to adrenaline. Preliminary experiments indicated that none of the suggested causes of the secondary effect, mentioned above, would offer a satisfactory explanation, the more so as they mostly apply to results obtained on

species other than the rabbit. An attempt has therefore been made to trace (1) the cause of the secondary fall in blood pressure after adrenaline in the rabbit, and (2) the mechanism of the abolition of this effect by small doses of sympathicolytic ergot alkaloids.

Experimental

The rabbits, mostly between 2·2 and 3·7 kg. in weight, were anæsthetized with urethane (6 c.c. 25 p.c. solution per kg.) or pernocton (0·6 c.c. 10 p.c. solution per kg.) intravenously, or ether. In some cases a combined anæsthesia of urethane and pernocton was given. The secondary fall appeared under all of the anæsthetics mentioned, as it does in the unanæsthetized animal. The blood pressure was recorded by means of a mercury manometer from the left carotid artery. Respiration was spontaneous unless otherwise stated.

The secondary fall in blood pressure after adrenaline

This frequently recorded phenomenon is shown in Fig. 1 *A*. The secondary fall varies in its extent, but has been absent only in a small minority of cases. The fall is generally well developed in the animal

Fig. 1. Rabbit, blood pressure, urethane. *A*, 0·04 mg. adrenaline i.v. Between *A* and *B* both depressor nerves cut and carotid sinuses denervated. *B*, 0·04 mg. adrenaline i.v. *C*, ice on both vagi in the neck, 0·04 mg. adrenaline i.v. *X*, drum stopped.

under urethane after 0·005–0·04 mg. adrenaline. In two rabbits, rather deeply anæsthetized with pernocton and subjected to artificial respiration, the secondary fall was missing, the blood pressure remaining above the previous level for some 5 min. after the injection of adrenaline (Fig. 2 *A*). On the other hand, it was observed that moderate asphyxia, as brought about by letting the animals rebreathe through a length of tubing, would increase the secondary fall (Fig. 2 *B*).

As a rise in blood pressure is known to elicit vasodilatation and slowing of the heart by reflex actions through the buffer nerves, it became necessary to examine the effect of adrenaline after exclusion of these reflexes. For this purpose the depressor nerves on both sides were cut in the neck and both carotid sinuses were denervated. This was effected by isolating the external and the internal carotid arteries for a distance of about 5 mm. from the bifurcation, the tissue between the vessels being divided between ligatures. It was noted, however, that under urethane anæsthesia occlusion of the second common carotid, after denervation in the way described, caused in some cases a considerable rise in blood pressure, which could not be explained as a mechanical effect only. In these cases the same rise was obtained by clamping the

Fig. 2. *A*, rabbit, blood pressure, pernocton. Artificial respiration. Sinus and depressor nerves cut. 0·01 mg. adrenaline i.v. *B*, rabbit, blood pressure, urethane, sinus and depressor nerves cut. 0·02 mg. adrenaline i.v., before and after increase of respiratory dead space. *X*, drum stopped.

external carotid, and it is believed that this effect is reflex in origin and not merely mechanical, since the clamping of other vessels of the same size does not produce a similar rise. It could be shown, furthermore, that clamping the branches of the external carotid also caused a considerable rise in pressure, whereas occlusion of the internal carotid was without effect. By partial occlusion of the external carotid different degrees of rise in general blood pressure could be obtained. The site of the presumptive area of origin of these reflexes, peripheral to the carotid sinus, was not further investigated. In order to exclude the reflex changes in blood pressure, due to variations in pressure in this barosensitive region, the external carotids on both sides were tied in cases where this effect occurred. Variations in pressure in the common carotid were now either without any distinct action on the general blood pressure, or followed by insignificant changes only. Traction on the common carotid

after denervation of the sinus still produced a considerable fall in blood pressure, which effect could also be evoked by pinching or even light touching of the carotid artery in several cases. This effect is clearly not related to the sinus reflex proper. It seems that this effect is due merely to the stimulation of sensory nerves in the arterial wall. It has been pointed out by Florey & Marvin [1928] that the rabbit under urethane readily responds with a fall of blood pressure to stimuli of very various kinds, and this observation has been amply confirmed by the present writer. There is no evidence, however, that such changes in blood pressure as are brought about by the doses of adrenaline employed would cause any reflex change of the general blood pressure, after denervation of the carotid sinuses and cutting the depressor nerves in the manner described.

Fig. 3. Rabbit, blood pressure, urethane. *A*, 0·005 mg. adrenaline i.v. Between *A* and *B*, 5 mg. atropine sulphate. *B*, 0·005 mg. adrenaline i.v. *C*, about 1 hr. later, 0·005 mg. adrenaline i.v.

After exclusion of the barosensitive areas, including the tying of the external carotid arteries, the secondary fall after adrenaline still persisted (Fig. 1 *B*), though the effect in some cases seemed to have been diminished. It was thus clearly established that the effect in question was not due, at least not chiefly, to reflex actions originating in the barosensitive areas.

In order to determine whether the secondary fall is due to a central or a peripheral effect of adrenaline a search was made for possible nerve paths, transmitting impulses resulting in a fall in pressure. It was thus found that section of both vagi in the neck abolished the effect. This could be shown also when a temporary break was made in the nervous conduction by placing a piece of ice on the isolated vagi in the neck (Fig. 1 *C*). On removing the ice and allowing the nerve conduction to be restored the effect reappeared.

The secondary fall is therefore, as it appears, entirely dependent on impulses conducted through the vagi. Since it was found that injection

of atropine also prevents the effect (Fig. 3) afferent impulses in the vagi are not alone involved, if at all, and the effect must be due to an efferent function of the vagus fibres. This implies that the effect cannot be attributed to a diminution of the central vasomotor tone, but must be due to some actively depressor impulses transmitted by the vagi.

Effect of stimulation of the vagi below the heart. Actions of atropine, eserine and ergotoxine

Since the secondary fall after adrenaline, shown to be prevented by section of the vagi in the neck or by atropine may be due to a decreased output of the heart, or peripheral vasodilatation, or both, it seemed

Fig. 4. Rabbit, blood pressure, pernocton. Sinus and depressor nerves cut. *A*, 0·01 mg. adrenaline i.v. Between *A* and *B* both vagi cut intrathoracically below the heart. *B*, 0·01 mg. adrenaline i.v. Between *B* and *C* both vagi cut in the neck. *C*, 0·01 mg. adrenaline i.v. *X*, drum stopped.

desirable to determine whether and to what extent the heart was involved. An attempt was made to cut the vagal branches to the heart in the chest, leaving the rest of the vagus intact, but this was found to be technically impracticable in the rabbit. Both vagi, therefore, were prepared on the œsophagus below the heart, with an open chest, and divided. This was found greatly to reduce the secondary fall after adrenaline, though division of the vagi in the neck decreased it yet further, or abolished it (Fig. 4). These results show that the effect in question is partly transmitted by the vagi below the heart and is partly due to an action transmitted by vagus fibres ending at a higher level, presumably in the heart. As to the former effect, it seemed reasonable to assume that it was due to vasodilator impulses carried by the vagi. Since the vagi may not improbably contain vasodilators for the splanchnic area and since the vascular bed of this region has a relatively large

significance in the rabbit, the blood pressure might readily be affected by vasodilator impulses carried to this area through the vagi.

The peripheral ends of the vagi, after division in the chest below the heart, were stimulated by faradization. In all cases a drop in blood pressure ensued, though the fall showed some variations in shape and extent. In two out of six cases the fall set in almost immediately and continued for the time of stimulation (20–40 sec.). After the stimulation had ended, the blood pressure returned to normal within a short time (Fig. 5 *A*). In the remaining cases the blood pressure fell more slowly, remained low for some time, and then recovered gradually (Fig. 5 *B*). In some instances a preliminary short rise preceded the fall. The drop in blood pressure during stimulation in some cases exceeded 35 mm. Hg. (In the cat the stimulation of the vagi below the heart causes, on the contrary, a small rise in blood pressure as observed by Brown & Garry [1932]. The same result was obtained in a single experiment on the cat by the present writer.)

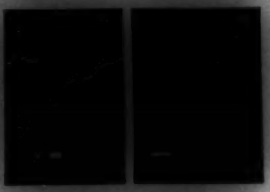

Fig. 5. Rabbit, blood pressure, pernocton. *A*, peripheral ends of vagi stimulated intrathoracically below the heart for 10 + 25 sec. *B*, another animal, same stimulation for 40 sec.

Fig. 6. Rabbit, blood pressure, pernocton. *A*, peripheral ends of vagi stimulated intrathoracically below the heart 20 sec. at 12.50. At 12.58 4 mg. atropine sulphate i.v. *B*, at 1.02 and 1.03 p.m. stimulation 20 sec. *C*, at 1.40 stimulation 20 sec. *D*, at 2.34 stimulation 25 sec. Between *D* and *E* both vagi tied below the diaphragm. *E*, at 2.45 stimulation 30 sec.

We have seen that the secondary fall in blood pressure after adrenaline was abolished by atropine (Fig. 3). It seemed, therefore, of interest to examine the action of atropine on the fall in blood pressure caused by stimulation of the peripheral ends of the intrathoracic vagi as described above. After vagus stimulation had been shown to produce a definite fall in blood pressure, 4 mg. of atropine were injected intravenously, and

caused a transient, moderate fall in blood pressure. As soon as the pressure had recovered to a steady level, the stimulation of the vagi was repeated and now no longer caused any significant fall. After about 1½ hr. the response to stimulation had returned, being now practically the same as before the administration of atropine (Fig. 6).

For the mechanism of this fall in blood pressure during vagus stimulation, several possibilities arise. A spread of current to sensory nerves, the stimulation of which generally causes a drop in blood pressure in the rabbit under urethane or pernocton, was ruled out by the facts that tying the vagus nerves below the diaphragm prevented the development of the fall, and that the effect was abolished by atropine. A diminution of the blood supply to the heart, as a result of contraction of the diaphragm, which was occasionally observed, could be excluded for the same reasons. The same argument might be put forward for any type of action not due to change in the activity of plain muscle innervated by the vagi. There seems to be no way of discriminating, however, between a true vasodilator effect and other effects on the distribution of the blood within the splanchnic area, arising from the activity of plain muscle, which might for one reason or other diminish the flow of blood to the heart. On the other hand, it is the general experience that atropine does not abolish the motor effects of vagus stimulation on the intestine of the rabbit. Reviewing the evidence, it seems fair to assume that the fall in blood pressure, during subcardial vagus stimulation in the rabbit, is due to vasodilatation in the splanchnic area. This would also account for the diminution of the secondary fall after adrenaline, when the vagi are divided below the heart, or atropine is administered.

The effect of centrally injected adrenaline on the blood pressure

Whereas adrenaline may increase the blood supply to the centres by raising the general blood pressure, and may thus remove a high vasomotor tone due to partial asphyxia [Nowak & Samaan, 1935], it may cause, under somewhat different conditions, a central depressant effect on the blood pressure which can hardly be explained in this way. Thus Dale & Richards [1927] observed that, while small intravenous doses of adrenaline would cause a moderate fall in blood pressure in the dog owing to a peripherally excited vasodilatation, similar doses, when injected into an artery supplying the centres, caused a much more powerful depressant action. In their experiment the dog's vagi were cut, the left subclavian artery tied centrally to the origin of the vertebral artery, all branches of the right subclavian artery except the vertebral

were tied, and a cannula was inserted in the central end of the right subclavian artery, enabling injections to be made through the right vertebral artery towards the centres. Both common carotids were tied. A rather spectacular fall in blood pressure followed the central injection of 0·005 mg. adrenaline by this route. Since adrenaline is known to elicit constriction of the cerebral vessels [Bouckaert & Jourdan, 1936] it seems likely that, in the experiment of Dale & Richards, the "abnormal" depressor effect was due to acute anoxia in the vasomotor centre, which would cause an instantaneous depression of the vasomotor tone.

Some similar experiments have been carried out in the present investigation in order to examine the central effects of adrenaline on the blood pressure of the rabbit. In a number of experiments on rabbits under various anæsthetics and in one experiment on the cat under chloralose, the adrenaline was injected through the internal carotid artery on one side, the second carotid being tied. A small dose, 0·001–0·002 mg., of adrenaline under such conditions regularly caused a transient pure fall in blood pressure, which was not followed by a rise after these amounts. The effect was greatly enhanced by cocaine (Fig. 7). It was tempting to assume that this effect of adrenaline was analogous to the delayed, secondary depressor effect observed after an intravenous injection of adrenaline. It could be shown, however, that this was not the case. Section of the vagi in the neck did not appreciably diminish this effect of the central injection of adrenaline and it was not abolished by atropine. The effect could therefore not be caused by a stimulation of the vasodilator or cardio-inhibitory centres (vagus centre), but must probably be due to a diminution of the vasomotor tone. Since the injection of adrenaline causes vasoconstriction and the observed effect is enhanced by cocaine, the central depression will obviously be best explained by assuming an acute anoxia in the vasomotor centre, causing a "shock"-like fall in blood pressure. The readiness with which this effect of adrenaline can be evoked under certain conditions appears to be of a certain interest for the production of sudden falls in blood pressure, and does not seem to have been explained earlier on these lines.

Fig. 7. Rabbit, blood pressure, ether. *A*, 0·002 mg. adrenaline into the internal carotid artery. *S*, same as *A* after 0·5 mg. cocaine hydrochloride injected the same way about 20 min. earlier.

The fact that anoxia gives rise to a fall of rapid onset in general blood pressure has been shown for the cat by Euler & Liljestrand [1936], who observed that the fall commences before the respiration is affected. The anoxia was produced by letting the animals breathe mixtures of oxygen and nitrogen. Hyperventilation did not occur since the carotid sinuses were denervated. In the present experiments anoxia was produced experimentally in the same way. Even if the anoxia was moderate, so as to evoke no marked change in the respiration of the animal deprived of its sinus innervation, a fall in blood pressure was observed (Fig. 8), which persisted after section of the vagi in the neck, though in some cases it appeared to be diminished. This may be explained as a mixed effect of the anoxia: partly due to a stimulation of the vagus centre and partly to a slight depression of the vasomotor centre. Peripheral effects due to accumulation of acid metabolites could not, however, be excluded in these experiments.

Fig. 8. Rabbit, ether. Upper curve, respiration; lower curve, blood pressure. Between arrows the rabbit was breathing a mixture of 8 p.c. oxygen in nitrogen. Sinus and depressor nerves cut.

Central anoxia was also produced in some experiments by injections of small doses of potassium cyanide directly towards the centres. These injections were also followed by a transient fall in blood pressure, much of the same type as those produced by adrenaline administered by the same route, and, like these, persisting after vagotomy. These experiments apparently support the explanation of the central depressant action of adrenaline suggested above.

It is interesting to note that, whereas a gradually developed anoxæmia, in the cat deprived of its sinus innervation, causes a fall in blood pressure, the same condition in the dog produces a rise in pressure, as observed by Euler & Liljestrand. In the rabbit I found a more complex action, the initial effect being a fall, which was in some cases followed by a transient rise in pressure which then was succeeded by a fatal fall. It seems, therefore, that the sensitiveness and the mode of reaction of the vasomotor centres to anoxia differs substantially in different species.

Abolition by ergotoxine of the secondary fall after adrenaline

As already mentioned, it was observed that intravenous injection of the sympathicolytic ergot alkaloids in doses of the order of 0·05–0·1 mg. per kg. rabbit suppressed the secondary fall after adrenaline, without causing any marked alteration of the primary, pressor effect (Fig. 9). In cases where the secondary fall was increased by moderate asphyxia (rebreathing), this effect was also abolished by ergotoxine or ergotamine. It could be shown further that this effect is not peripheral, since an amount known to be sufficient to abolish the secondary fall does not prevent the fall in blood pressure obtained after stimulation of the peripheral ends of the vagi below the heart. These results are in

Fig. 9. Rabbit, blood pressure, urethane. *a*, 0·02 mg. adrenaline i.v. *b*, 0·2 mg. ergotoxine ethanesulphonate i.v. *c*, 0·02 mg. adrenaline i.v. *X*. drum stopped.

accordance with the early observations on the action of ergot by Dale [1906], who stated that "the cranial and sacral autonomic inhibitory nerves to the heart and arteries suffer no paralysis at any level". According to Rothlin [1923], who was the first to find that the depressor reflex is abolished by ergotamine, the excitability of the vagi is increased after ergotamine.

Using small doses of ergotamine (0·1–0·15 mg. per kg. body weight) Wright [1930] observed that the depressor and sinus reflexes in the vagotomized cat under chloralose were abolished, and concluded from these experiments and from the results of cerebral anæmia, which still produces a rise in pressure, that ergotamine exerts a paralysing effect on the afferent side of the vasomotor centre. With larger doses of ergotamine the pressor sinus reflexes as well were affected, and finally abolished. As pointed out by Heymans *et al.* [1930], the fact that cerebral anæmia still produces a rise in blood pressure

after a small dose of ergotamine may depend on the difference in type of stimulus. The last mentioned authors have also shown that ergotamine may abolish vasomotor reflexes by a central action, though apparently only pressor reflexes were studied. The effect of ergotoxine was also examined on the central depressant action of adrenaline, given by central arterial injection. It was found that this was diminished but not abolished by a dose of 0·005–0·01 mg. ergotoxine injected towards the centres, though scarcely affected by a dose of 0·15 mg. injected by a vein into the general circulation. This confirms the view that the secondary fall after intravenous adrenaline and the depressor effect of centripetal, arterial injection are not identical in their causation, as already suggested; small doses of ergotoxine apparently do not abolish the depression of the vasomotor centre evoked by anoxia.

Discussion

The secondary fall in blood pressure after adrenaline in the rabbit is evidently due to action on the centres, no evidence having been obtained either for a reflex action or a peripheral action. Brown [1916] produced evidence that adrenaline, injected into an isolated cerebral circulation, caused stimulation of the vagus centre. As to whether adrenaline has a direct action on the cells in the nerve centres or acts only through effects on the cerebral vessels, Nowak & Samaan [1935] have recently given reasons for believing that the central depressor effect sometimes observed in the dog is due to the latter cause, thus confirming the opinion held by Heymans [1933]. Nowak & Samaan, in experiments in which the head of the dog under observation was perfused by crossed circulation from another dog, found that adrenaline injected into the circulation of the donor would cause a general vasodilatation as shown by a fall in the blood pressure of the recipient, especially in those cases where the recipient vasomotor centre had an already restricted blood supply, and a consequent persistent increase in its tone, due to partial asphyxia. They argued that, in such circumstances, adrenaline injected into the circulation of the donor, increasing the blood supply of the isolated head by raising the pressure under which it was perfused, would remove the asphyxial stimulus from the vasomotor centre and thus cause vasodilatation and fall in blood pressure. While an increased perfusion pressure by itself would doubtless increase the blood supply to the centres, it appears less evident that adrenaline in all circumstances would produce this result. In view of the vasoconstrictor action of

adrenaline on the cerebral vessels [Bouckaert & Jourdan] it seems conceivable that adrenaline might cause its central effects also by diminishing the blood supply to the centres. According to Yamakita [1922] intravenously administered adrenaline causes a diminished blood flow through the centres, lasting for several minutes. Theoretically the following events may happen as a result of diminished blood supply: (1) stimulation by partial asphyxia of centres which act upon the blood pressure, and (2) depression of the same centres, due to a more severe anoxia. Since, in either case, centres with a depressor or a pressor effect may be affected, the final result will depend (1) on the centre predominantly affected, and (2) the degree of asphyxia or anoxia. In this connexion the question presents itself, whether, with regard to centres with a depressor effect, the actions are brought about by an influence on primary or secondary centres. If a vasodilator effect is transmitted through the vagi, as in the case of the secondary fall after adrenaline, it cannot at present be stated whether the effect originates at a primary centre, or arises at the vagus centre itself. The fact that cutting the vagi in the neck prevents the secondary fall after adrenaline does not exclude the former possibility, however, since the vagi may carry practically all the vasodilator fibres concerned in the rabbit.

The effect of a small dose of ergotoxine in my experiments is to annul the normal output of depressor impulses in the vagus, following the adrenaline rise of blood pressure, in the rabbit. This effect on the vagus centre might be due to a weak persistence in its vessels of the constrictor action of adrenaline, after its subsidence elsewhere: in that case the effect of ergotoxine might be due to a suppression of this vasoconstrictor remainder. On the other hand, on the analogy of the experiments of Wright, the effect of ergotoxine may be due to block of conduction at synapses correlated with the centres; in that case the secondary fall following the adrenaline rise would presumably be due to action, not on the vagus centre itself, but on some centre making synaptic connexions with it. My evidence, however, would not justify an attempt to choose among such possibilities.

Reviewing the evidence, it seems most likely that the secondary fall is due to an increased activity, excited directly or indirectly, of the vagus centre, caused by vasoconstriction. If, on the other hand, a stimulating action on the vasomotor centre in the rabbit is set up for the same reason, the effect on the centre sending out depressor impulses must outlast this, giving a fall of pressure as the late result.

SUMMARY AND CONCLUSIONS

The secondary fall in blood pressure after moderate intravenous doses of adrenaline in the rabbit persists after denervation of the carotid sinuses and cutting of the depressor nerves.

The effect is suppressed by division of the vagi in the neck and abolished by atropine.

The effect is explained as being caused by stimulation of centres governing vasodilator and cardio-inhibitory effects, due to restriction of the blood supply by vasoconstriction.

Stimulation of the peripheral ends of the vagi intrathoracically, below the heart, causes a fall in blood pressure in the rabbit, which is abolished by atropine, enhanced by eserine and not affected by small doses of ergotoxine.

Small intravenous doses of ergotoxine or ergotamine (0·05–0·1 mg. per kg. rabbit) suppress the secondary fall after adrenaline, probably by a central depressant action on the vagus centre.

Arterial injection of adrenaline or potassium cyanide to the centres produces a fall in blood pressure, which is not prevented by section of the vagi or by atropine. The same effect is brought about by severe anoxia and is explained as due to depression of the vasomotor tone. This effect is not abolished by small injections of ergotoxine into the general circulation, but centrally injected ergotoxine may suppress the effect on adrenaline directed to the centres, by antagonizing its action on the cerebral vessels.

I wish to express my thanks to Sir Henry Dale for his stimulating interest in the work.

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THE EFFECT OF HYPOPHYSECTOMY ON THE BLOOD CALCIUM AND PHOSPHORUS OF THE RAT

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The presence of a parathyrotropic hormone in the pituitary was suggested by Anselmino *et al.* [1933, 1934], and by Hertz & Kranes [1934] on histological evidence of parathyroid hypertrophy in rats and rabbits after the injection of anterior pituitary extract.

A rise of 10–15 p.c. in the blood calcium in rats and dogs after the injection of anterior pituitary extract has been reported by Hoffmann & Anselmino [1934], and a rise in both the blood calcium and phosphorus has been reported in dogs by Gerschman & Marenzi [1935]. Shapiro [1934] found an increase of over 40 p.c. in the serum calcium level of cats, 5 hours after the injection of "Antuitrin". Friedgood [1936] and Friedgood & McLean [1937] have reported a rise in the blood calcium in rats and guinea-pigs after several daily injections of anterior pituitary extract.

If a rise in the serum calcium is found after injection of anterior pituitary extracts, it might be expected that a fall would result after hypophysectomy. This has been reported in the toad [Charles, 1931; Hogben *et al.* 1931; Shapiro & Zwarenstein, 1933], but it does not appear to occur in mammals. Collip [1934] reported that the serum calcium of the hypophysectomized rat was normal or slightly increased. Wallace [1935] also found normal values for the blood calcium in the hypophysectomized rat. Marenzi & Gerschman [1934] studied six dogs at times varying from 12 to 297 days after hypophysectomy, and could find no alteration either in the blood calcium or phosphorus.

Houssay [1936] also found no changes in the plasma calcium of forty-seven hypophysectomized dogs. Kusunoki [1927], on the other hand, described a striking increase in the serum calcium after hypophysectomy in the rabbit, although Cannavo & Beninato [1935] reported that there was no change in the blood calcium of rabbits after irradiation of the pituitary sufficient to produce severe damage in the anterior lobe.

In the foregoing experiments, where observations were made on the serum calcium or inorganic phosphorus after hypophysectomy, no details have been given of the dietary intake of calcium and phosphorus. Shelling [1932] showed that the calcium and phosphorus of the diet was a factor determining the level of serum calcium and the onset of tetany in parathyroidectomized animals. It seems necessary, therefore, to control the amount of calcium and phosphorus in the diet of hypophysectomized animals, before it is justifiable to claim that hypophysectomy has no effect on the parathyroids as indicated by the serum calcium and the plasma phosphorus levels. The experiments to be described were undertaken with this consideration in view.

Methods

The animals used were exclusively males of the London strain of Wistar rat. At ages of from 8 to 12 weeks they were hypophysectomized under ether anæsthesia, using the technique of Smith [1927] with slight modifications. The testicular atrophy formed a convenient sign in the living animal of the completeness of the removal of the hypophysis, which was also shown by cessation of growth. More than 80 p.c. of the animals survived the operation, a few dying some weeks later in hypoglycæmic coma. Control animals of the same age and, where possible, litter mates, were kept on the same diet and under identical conditions.

Blood was drawn by heart puncture under ether anæsthesia, the animal killed, and the testes examined. Marked atrophy of the testes of the hypophysectomized animals was always found.

Calcium was determined in the serum by Clark & Collip's [1925] modification of Kramer & Tisdall's method.

As the rats yielded only small quantities of blood, it was not possible to estimate both calcium and inorganic phosphate in the blood of a single rat. The plasma inorganic phosphate was therefore estimated in a second series of animals by the method of Briggs [1922]. The blood was drawn as described above, oxalated and centrifuged immediately.

Results

The preliminary experiments were carried out on animals fed on a stock diet containing 30 p.c. of dried milk, with occasional change to bread and fresh milk. The animals were killed at periods varying from 14 to 70 days after operation. The serum calcium of ten hypophysectomized animals varied from 9·3 to 11·8 mg./100 ml. with a mean of 10·6 mg., and that of eight controls varied from 9·6 to 11·3 mg./100 ml. with a mean of 10·3 mg. Therefore hypophysectomy had no effect on the serum calcium of rats fed on the stock diet.

Animals were next given a low calcium diet of the same composition as one used by Shelling [1932] and designated I–K in his paper. In his experiments parathyroidectomized rats fed on this diet developed tetany and showed serum calcium levels of 7·2–2·5 mg./100 ml. According to Shelling the diet contained calcium 0·012 g./100 g., and phosphorus 0·475 g./100 g. Our diet prepared with a purified casein (Glaxo ashless) gave on analysis calcium 0·032 g./100 g. Rats fed on this diet for a few weeks in our experiments developed a considerable degree of osteoporosis, which was evident at operation when the bone at the base of the skull was found to be very soft and vascular, in contrast to the hard dry bone of the normal rat. This osteoporosis was also shown by skiagrams of the rats taken after death. The results obtained with this diet are given in

Table I. Rats fed on diet containing calcium 0·032 g./100 g.

Hypophysectomized				Controls			
No. of rat	Days after operation	Days on diet	(A) Serum calcium mg./100 ml.	No. of rat	Days on diet	(B) Serum calcium mg./100 ml.	Difference (A) and (B)
79*	17	17	9·2	78*	19	9·2	0
83	17	17	9·7	77	19	9·5	+0·2
63*	31	31	9·3	69*	31	6·5	+2·8
89, 90	32	32	8·8	94	32	10·1	−1·3
82	37	37	9·3				
74*	22	40	8·1	75*	40	6·4	+1·7
73	22	40	8·3	76	40	4·9	+3·4
68*	13	45	7·5	71*	45	5·2	+2·3
70	13	45	5·9	72	45	5·8	+0·1
84	31	45	9·8	85	45	10·4	−0·6
86	31	45	10·0	93	45	9·8	+0·2
101	24	47	6·7	102	47	5·7	+1·0
103	24	47	7·4	104	47	5·3	+2·1
				105	49	6·3	
				106	49	5·6	
				98	57	6·8	
				99	57	7·1	
				Mean difference (A) and (B)			+0·99

* Thyroxine 3 mg./kg. injected at 10 and 5 days before blood drawn.

Table I, which shows that the serum calcium of the control rats fell to low levels in most cases where the rats had been on the diet for 40 or more days. None of these animals was in actual tetany, but many showed muscular hyperirritability. The serum calcium was significantly higher in the hypophysectomized animals than in the controls. It was considered possible that the hypothyroid state of the rats after removal of the hypophysis would affect the calcium metabolism, and some of the animals—marked with an asterisk in Table I—were given thyroxine (B.D.H.) subcutaneously in doses of 3 mg./kg. at 10 and again at 5 days before blood was drawn. Control rats received similar injections. These doses were based on the work of Gaddum [1930] on the effect of thyroxine on the metabolism of the rat. The injection of thyroxine had no consistent effect on the serum calcium of either the experimental or control rats (Table I).

The inorganic phosphate was first determined in the plasma of a few hypophysectomized rats on the stock diet, where it was found to be lower than in control animals. In another series of animals fed on the low calcium diet, the inorganic phosphate of the plasma was estimated at periods varying from 7 to 42 days after hypophysectomy. As will be seen from Table II, the plasma inorganic phosphate of the hypophysectomized animals—with a mean value of 4·64 mg./100 ml.—was markedly lower than that of their controls—mean 7·46 mg./100 ml.

Table II. Rats fed on diet containing calcium 0·032 g./100 g.

Hypophysectomized				Controls		
No. of rat	Days after operation	Days on diet	Plasma inorganic phosphate mg./100 ml.	No. of rat	Days on diet	Plasma inorganic phosphate mg./100 ml.
148	7	7	4·5	150	7	6·1
149	7	7	5·5			
144	14	14	4·6	146	14	7·05
145	14	14	3·8	147	14	8·4
108	19	21	5·4	115	21	6·5
109	19	21	4·8	116	21	7·5
110	19	21	4·0	117	21	7·65
111	21	23	5·6	127	23	8·3
				128	23	7·75
				129	23	8·7
120	43	43	4·6	125	41	6·4
123	41	41	4·35	130	41	7·7
124	42	42	4·3	119	43	7·7
126	42	42	4·3	131	42	7·2
		Mean	4·64			7·46

Discussion

The histological evidence for the existence of a parathyrotropic hormone is not by any means conclusive.

The results recorded of the effect of hypophysectomy on the parathyroid are not in agreement. Atrophy of the parathyroids after hypophysectomy has been reported in rats by Smith [1927], and in dogs by Koster & Geesink [1928], but in neither case have any details been given as to the type or degree of the atrophy. Houssay [1936] has recorded changes after hypophysectomy in the parathyroids of dogs, which may be localized to one gland only, or to part of one gland, the remainder of the gland being normal. His results do not resemble those seen in other endocrine glands after hypophysectomy, where there is a generalized histological change after removal of the tropic influence. Houssay himself states that these changes may be the result of general nutritive alterations or the result of the lack of parathyrotropic hormone. On the other hand Collip [1935] could find no consistent degenerative changes in the parathyroids either of dogs or rats even several months after removal of their pituitaries. Livon & Peyron [1911] and Aschner [1912] also could find no changes in the parathyroids of dogs after hypophysectomy.

The other evidence adduced in support of a parathyrotropic factor, namely the changes in the blood calcium after injection of anterior pituitary extracts, is also inconclusive. Only comparatively crude extracts of the anterior pituitary have been used, and no separation of a parathyrotropic from the other factors has been obtained. The rises in the serum calcium after these injections in most cases were small, of the order of 10–15 p.c., and are not comparable to those found after injection of parathormone. Hogben & Charles [1932] found that a saline extract of the anterior lobes of ox pituitaries produced a marked fall in the serum calcium when injected into normal female rabbits. They also showed that this action was not through the ovaries since it occurred to the same degree after ovariectomy.

The results showing the effects of anterior pituitary extracts on the plasma phosphorus are also contradictory. Friedgood & McLean [1937] could find no significant changes in the level of the blood phosphorus in guinea-pigs after daily injection of anterior pituitary extract for 9 days. On the other hand Gerschman & Marenzi [1935] reported a rise in the plasma phosphorus in dogs after injection of anterior pituitary extract.

There is the possibility that the alterations recorded in the serum calcium may have been due primarily to a tropic factor other than a possible parathyrotropic hormone, since the anterior pituitary extracts used had not been freed from all the other factors. Such a factor may have acted through its respective endocrine gland directly on the serum calcium or indirectly through the action of its own endocrine gland on the parathyroids.

Our results show that hypophysectomy has no effect on the serum calcium of rats on a normal diet. This finding agrees with other results reported in the literature regarding the effect of hypophysectomy in mammals. In our experiments with a low calcium diet, it was found that after some weeks most of the control rats had a serum calcium of the order of 5–6 mg./100 ml. Hypophysectomy did not produce a lower serum calcium; in fact the calcium was higher in the serum of the hypophysectomized animals. Many of these rats had been fed on the low calcium diet for 2–3 weeks before operation, and therefore should have been particularly sensitive to any parathyroid deficiency. Shelling [1932] has shown the importance of this factor, because fatal tetany ensued within 12–24 hours after he had removed the parathyroids of animals which had been fed on this diet for a few days before operation.

The plasma inorganic phosphate of the rats fed on this diet was markedly lower after hypophysectomy. Whatever the explanation may be for our low figures for plasma phosphorus after hypophysectomy, they are in striking contrast to the raised plasma phosphorus found after parathyroidectomy. The diminished intake of food by the hypophysectomized animal may account in part for the fall in phosphate. There is the further complicating factor of cessation of growth in these animals. Perla & Sandberg [1936] have shown that, after hypophysectomy in the rat, not only was there a very much increased excretion of calcium but also of phosphorus. Pugsley & Anderson [1934] have also found a negative calcium balance in the rat after hypophysectomy, and noted that this was changed to a positive balance after the administration of growth hormone.

The lack of effect of hypophysectomy on the parathyroid function as shown in our experiments is in marked contrast to the effect seen on the other endocrine glands when their respective tropic factors are removed. It is difficult to accept the existence of a parathyrotropic hormone, the removal of which appears to have no effect on the functional activity of the parathyroids.

SUMMARY

1. Hypophysectomy had no effect on the serum calcium of rats fed on a normal diet.

2. After some weeks on a low calcium diet, control rats showed a fall in serum calcium to 5–6 mg./100 ml. Hypophysectomy did not produce a lower serum calcium, but the plasma inorganic phosphate of the hypophysectomized rats—with a mean value of 4·6 mg./100 ml.—was markedly lower than that of the controls—mean 7·4 mg.

3. In view of our results, and of the conflicting accounts in the literature, it is concluded that the existence of a parathyrotropic hormone is not proved.

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THE EFFECT OF CERTAIN HORMONES ON THE ACTIVITY OF THE UTERINE MUSCLE OF THE MOUSE

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Previous investigations have shown that the ovarian hormones play an important part in determining the sensitivity (i.e. the reactivity to the oxytocic hormone of the posterior lobe) and the spontaneous activity of the rabbit's uterus. The effects on sensitivity can be demonstrated both on the isolated uterus and in the intact animal. Experiments on some other species have failed to give similar results. In the guinea-pig, for example, the sensitivity and motility were not appreciably affected by these hormones [Bell & Robson, 1936].

Accordingly it seemed of interest to investigate the conditions in one of the smaller rodents. The experiments were performed on the mouse since data are already available for the action of the ovarian hormones on the isolated uterus of this animal [Robson, 1934*a*]. In particular it appeared necessary to determine whether œstrin would increase the sensitivity *in vivo* as it is known to do *in vitro*, and whether progesterone would show any effect on the motility and sensitivity as investigated in the intact animal.

Methods

The experiments were performed on mature mice. All the animals were spayed by the dorsal route, and the uterine contractions recorded 7 days afterwards. The animals were divided into four groups: (1) a control group of three uninjected mice; (2) six animals injected with œstrone only; (3) ten animals injected with œstrone and progesterone; (4) five animals injected with œstrone and testosterone.

The œstrone, which was administered to all animals in groups 2, 3 and 4, was injected in oily solution in six doses on the morning and evening

of the 3rd, 4th and 5th days after spaying, the total amount in each being 20 μg.

Progesterone was given in oily solution in six doses on the morning and evening of the 4th, 5th and 6th days after spaying, the total amount administered being 0·75 mg. in each of five mice, 1·5 mg. in each of two mice, and 3·0 mg. in each of three mice.

A total amount of 3 mg. of testosterone in oily solution was given to the mice of group 4. The hormone was administered in six doses on the morning and evening of the 4th, 5th and 6th days after spaying.

We are indebted to Messrs Schering and Co. for the supply of progesterone in oil (proluton), to Dr Miescher of Ciba Ltd. for the testosterone, and to Dr White of Parke, Davis and Co. for a sample of specially purified oxytocic hormone (purified "pitocin").

The animals were anæsthetized with chloralose (6–7 mg. in saline subcutaneously). The movements of the longitudinal muscle of the uterus were magnified by a photoelectric method and recorded on smoked paper by the method previously described for the guinea-pig [Bell & Robson, 1936]. The uterus was attached to the cannula and lever and the abdomen was closed. The body temperature was read by means of a thermocouple inserted with the cannula into the abdomen. By varying the distance between a reading lamp and a layer of cotton wool covering the animal the temperature could be kept steady at 37° C. Recording of uterine contractions was not begun until the temperature was within physiological limits. By maintaining the body temperature in this way the preparation could be kept in apparently good condition for 5 hours or more.

Oxytocin was injected into a tail vein in a constant volume of 0·1 c.c. saline. In all experiments an attempt was made to estimate the smallest amount of oxytocin needed to elicit a definite motor effect. This was taken as a measure of the sensitivity of the uterine muscle.

Vaginal smears were taken from all animals just before the recording of the contractions.

Results

The results showed that the spontaneous activity was small in group 1, but higher in groups 2, 3 and 4, though there was no appreciable difference in this respect between these three groups. It can, therefore, be concluded that administration of œstrone increases the motility of the uterus but that neither progesterone nor testosterone produces any inhibitory action.

The sensitivity, i.e. the reactivity to oxytocin, in the last three groups was greater than in the untreated spayed animals of the first

group. No significant difference in sensitivity between the animals of the last three groups could be recognized. It must, therefore, be concluded that treatment with œstrone increases the sensitivity of the uterine muscle to oxytocin but that neither progesterone nor testosterone causes any desensitization.

Fig. 1. Record of movements *in vivo* of the longitudinal muscle of the uterus of mouse, which had been injected with œstrone only. Response to injection of 0·002 unit of "pitocin" intravenously but not to 0·0005 unit. The average movement of the centre of the uterus was about 5·7 mm. The temperatures indicate the abdominal temperature shown by the thermocouple. Time intervals in minutes. There was an interval of 30 min. between the two parts of this tracing.

The dose of œstrone chosen was sufficient to produce full vaginal cornification in all animals of group 2; four of these animals showed full œstrous distension of the uterus and the organ was partly distended in a fifth animal. Partial inhibition of the vaginal reaction to œstrone was produced by progesterone in six out of the ten mice of group 3. In no animal of this group was a full œstrous distension of the uterus seen.

Discussion

In these experiments an attempt has been made to use the various hormones in such dosage as is needed to produce the changes occurring in the normal physiological cycle. Hence the mice were injected with

20 μg. of œstrone since this amount (about 200 units) must be given to bring about the full changes of œstrous [Marrian & Parkes, 1930]. Progesterone was given in amounts up to 1 mg. per day. This may seem a large dose, but since about 1 mg. is necessary to maintain pregnancy in a spayed mouse [Robson, unpublished data] it must be considered within physiological limits. That the dose of testosterone was more than adequate is suggested by the fact that much smaller doses will inhibit the œstrous cycle in mice [Robson, 1936].

The previous investigations on the rabbit [Robson, 1934*b*] have suggested that the alterations in the sensitivity of the uterine muscle during pregnancy and parturition can be explained in terms of the action of the two ovarian hormones, œstrin and progestin. Experiments on the uterus of the mouse *in vitro* have shown that the œstrous hormone increases the uterine sensitivity as in the rabbit [Robson, 1934*a*], and the present investigations demonstrate that this also holds for the uterus in the intact animal. Progestin does not desensitize the uterus of the mouse *in vitro* and this is not due to any discrepancy between the behaviour of the muscle in saline and in the intact animal as we now have shown that no desensitization occurs when the uterus is examined *in vivo*.

The effects of the ovarian hormones and of testosterone on the sensitivity, i.e. response to oxytocin, of the uterine muscle in various species have been studied by many workers. (Rabbit: Knaus, 1930; Robson, 1933*a*, *b*; Pompen, 1933; Makepeace, *et al.*, 1936. Guinea-pig: Siegmund, 1931; Bell & Robson, 1936. Mouse: Siegmund, 1931; Robson, 1934*a*; data in present paper. Rat: Siegmund & Kammerhuber, 1937. Cat: Robson & Schild, 1938. Bitch: Robson & Henderson, 1936. Human: Knaus, 1931; Moir, 1934.) An examination of their results reveals the remarkable diversity of the effects of these hormones on uterine sensitivity. Nevertheless, the fact remains that in the four species (rabbit, mouse, cat, guinea-pig) that have been so far quantitatively investigated, the sensitivity of the uterine muscle increases during pregnancy and reaches a maximum at parturition. Brooksby [1937] has shown that the response of the rat's uterus *in vitro* to oxytocin does not increase up to the 20th day of pregnancy, but unfortunately no observations on the condition at parturition were made. In some of these species the increase of sensitivity can be adequately explained by the action of the two ovarian hormones, although only in one (i.e. the rabbit) is the luteal hormone known to produce any effect on the sensitivity of the uterus. Consideration of the action of the ovarian

hormones on the motility of the uterus in different species reveals divergencies and difficulties of a similar kind [see Reynolds, 1937].

In view of the variations of the effects produced by these hormones the possibility must be considered that the same end effect on the sensitivity of the uterus at parturition may be brought about in different species by different hormone mechanisms.

Summary

The activity of the uterine muscle of the mouse has been studied *in vivo* in four groups of animals: (1) spayed and uninjected; (2) spayed, then treated with œstrone; (3) spayed, then injected with œstrone and progesterone; (4) spayed and injected with œstrone and testosterone.

In the first group the response to oxytocin (i.e. sensitivity) and the spontaneous activity were small. In the last three groups the sensitivity and the spontaneous activity were greater than in the first group, but there was no appreciable difference between these three groups in these respects. Neither progesterone nor testosterone has any modifying effect on the activity of the œstrone-treated uterus.

The effects of the ovarian hormones and of testosterone on the sensitivity of the uteri of different animals is discussed in relation to the variations of sensitivity during pregnancy and parturition.

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THE EFFECT OF THE PLACENTA ON THE BODY WEIGHT OF THE MOUSE

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It has previously been shown by one of us [Newton, 1935] that if the fœtuses of the pregnant mouse are destroyed about two-thirds of the way through pregnancy, the placentæ are retained for some days and delivered at a time corresponding closely to normal full-term. This delivery was called, for convenience, "pseudoparturition", and was accompanied by a considerable loss of weight which could not be accounted for. The explanation of this loss forms the subject of the present investigation, and the ultimate point at issue is whether or not the placenta can be shown to have an endocrine function.

The facts previously established were that on fœtal destruction there was no appreciable loss of weight, and that 24 hours or so afterwards fœtuses and amniotic fluid had disappeared from the uterus, which had contracted down on the attached placentæ. The weight of the placentæ was insufficient to account for more than a fraction of the weight loss subsequently observed to follow their disappearance (Fig. 1). It will be recalled that the presence or absence of the placentæ is determined by daily palpation under ether anæsthesia, and that unless special precautions are taken they are never actually seen, but are eaten by the mother.

These observations called for an investigation of food metabolism and water balance, special attention being paid to (*a*) the constancy or otherwise of the metabolic rate of the normal non-pregnant animal,

(*b*) any abrupt change associated with the change from the non-pregnant to the pregnant state, (*c*) the metabolic rate during pregnancy, and (*d*) metabolic changes associated with pseudoparturition.

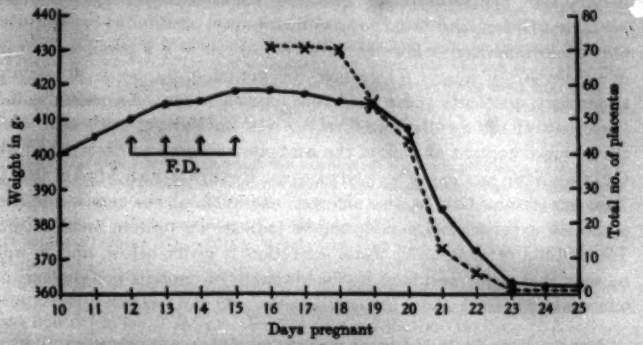

Fig. 1. Total weight (continuous line) and total number of placentæ (broken line) in fifteen mice from the 10th and 16th days respectively to the 25th day of pregnancy. Fœtal destruction (F.D.) was performed on the days indicated. (From Newton, 1935.)

Methods

Four closed metabolism chambers [Newton, 1937] were employed simultaneously, each containing a single mouse. The animals were weighed and put into the chambers at 4.30 p.m. each day, and at 9.30 a.m. the next morning were weighed again and removed to their cages in the same room. They were therefore free for 7 hours out of the 24, during which interval they had access to an unlimited supply of food and water. The urine was always expressed from the bladder before weighing. Mice under experiment for as long as 34 days showed no loss of weight or ill effects from this treatment, and the advantages of a long observational period in an animal whose metabolic rate is so notoriously inconstant are obvious. Moreover, in these experiments, a considerable loss of water was suspected at pseudoparturition, and it was necessary in order that this should be detected to have the animals under controlled conditions for the greater part of the day.

Chevillard [1935] found that mice with free access to food and water throughout the 24 hours ate chiefly within a few hours of receiving fresh food. Their weight, as a result, rose through 6–12 p.c. of its initial value in 6–8 hours, and then fell steadily, returning to its original level at the time of administration of fresh food 24 hours later. It seems, therefore, that our treatment of the mice is more physiological than might at first appear.

The oxygen usage and the carbon dioxide production were measured in the way previously described, but in order to determine the water excretion it was necessary to have an estimate of the nitrogenous metabolism. The measurement of water excretion was required to be accurate to 0·1 g., and if the gaseous metabolism is assumed to represent only the combustion of fat and carbohydrate, there is a possible error in one direction of nearly 0·1 g. over the period of 17 hours at certain respiratory quotients and a moderate oxygen usage. Accordingly, the lower half of the metabolism chamber was washed out with acid, and the nitrogen content of such of the contents as dissolved determined by the gasometric method of van Slyke [see Peters & van Slyke, 1932]. This was assumed to be the nitrogen excretion of the mouse for the preceding experimental period, and to indicate its protein metabolism. The method of computing water excretion is given below, and it will be seen that great accuracy in the estimate of protein metabolism is unnecessary:

Example. Mouse 24. 11–12 June. O_2 consumption, 1316 c.c.; CO_2 production, 976 c.c.; protein metabolized, 0·21 g.; R.Q. 0·74.

	O_2 consumption by protein	= 202·00 c.c.
	CO_2 production by protein	= 162·00 c.c.
∴	O_2 consumption by fat and carbohydrate	= 1114·00 c.c.
and	CO_2 production by fat and carbohydrate	= 814·00 c.c.
	Non-protein R.Q.	= 0·73
∴	weight of fat metabolized	= 0·50 g.
and	weight of carbohydrate metabolized	= 0·13 g.
and	weight of total food metabolized	= 0·84 g.
Now,	weight of mouse before entering chamber	= 22·60 g.
and	weight of mouse after leaving chamber	= 19·90 g.
∴	loss of weight of mouse	= 2·70 g.
and	loss unaccounted for by food metabolism	= 1·90 g.

If the protein be neglected, and the total weight of food metabolized assumed to be fat and carbohydrate, being computed from the gross R.Q. and total oxygen usage, it amounts to 0·78 g., the error being 0·06 g. If we take hypothetical examples, altering the CO_2 production in this experiment to give non-protein R.Q.'s of 0·7 and unity respectively, we find the corresponding errors to be 0·07 and 0·04 g. It is therefore advisable at low R.Q.'s to have an approximate value for nitrogen excretion, though this need not be very accurate.

The value of 1·9 g. derived above represents the water excreted (over and above that formed directly or by oxidation from the foodstuffs), the dry weight of the faeces and certain mineral constituents of the urine. Most of the urinary solutes (by weight) are protein derivatives, and are therefore accounted for in the "weight of protein metabolized", which has already been deducted from the total loss in weight of the mouse.

There is another way of arriving at the required figure which makes this point clearer. The gain in weight of the metabolism chamber overnight was, to the nearest 0·1 g., 4·6 g.

This must represent CO_2, total water, solid urinary constituents, and (dry) fæces excreted by the mouse overnight. Deduction of the weight of CO_2 (1·9 g.) leaves 2·7 g. Now, 1 g. carbohydrate, after oxidation of the carbon of the molecules, gives rise to 0·56 g. water, while 1 g. fat produces 0·95 g. water by oxidation and 0·14 g. from the residue of the molecules: 1·09 g. altogether. Deducting amounts corresponding to 0·13 g. carbohydrate and 0·5 g. fat (0·07 + 0·55 g.) leaves 2·1 g. Now, 1 g. protein produces 0·34 g. water by oxidation, after which (subtracting weights of carbon and hydrogen removed from the molecules) a residue of 0·57 g. is left. This is not all water, as in the case of the other two foodstuffs, but it is nevertheless excreted into the chamber partly as water and partly as urinary solutes, and must be deducted, i.e. a total of 0·91 g. for every gram of protein. The weight corresponding to 0·21 g. protein is 0·2 g., which on subtraction from 2·1 g. leaves 1·9 g.

The actual measurements on which this calculation depends are the same as those used for the simpler calculation, so that the two do not check each other, but are merely alternative ways of arriving at the same result. The assumptions made are also the same, namely, that 1 g. protein uses 960 c.c. O_2 and produces 770 c.c. CO_2; 1 g. fat uses 2020 c.c. O_2 and produces 1430 c.c. CO_2; and 1 g. carbohydrate uses 830 c.c. O_2 and produces an equal volume of CO_2.

The weights of the solid constituents of the fæces and sodium chloride, etc., of the urine, are small compared with 1·9 g. and similar weights, and are presumably of the same order from day to day. They have, therefore, been neglected, and the figure 1·9 g. is taken as fairly representing, for comparative purposes, the weight of ingested water or body fluid excreted.

The metabolism chambers (which take the temperature of their surroundings) were kept in a room whose temperature was controlled so as not to fall below 70° F., but in hot weather the temperature frequently rose above this figure. The use of the metabolic rate in computing water excretion, or accounting for loss of tissue, requires only that its actual daily value should be known, not that it should be constant. It will be seen from the results, however, that the conditions were sufficiently well controlled to ensure that the metabolic rate, though not basal, was sufficiently constant from day to day to enable us to detect any significant variations which might in themselves be important.

Mated mice were examined every morning for seminal plugs, and when these were found, the midnight following was assumed to mark the end of the first day of pregnancy.

Results

Records from seven of the experimental animals are shown in Figs. 2–4. The weight of each mouse has been plotted as a continuous line. The horizontal distance between each peak and its succeeding trough represents the experimental period of 17 hours, while the vertical distance represents the total weight lost during this period. The vertical distance between each peak and the first circle on the downstroke shows the

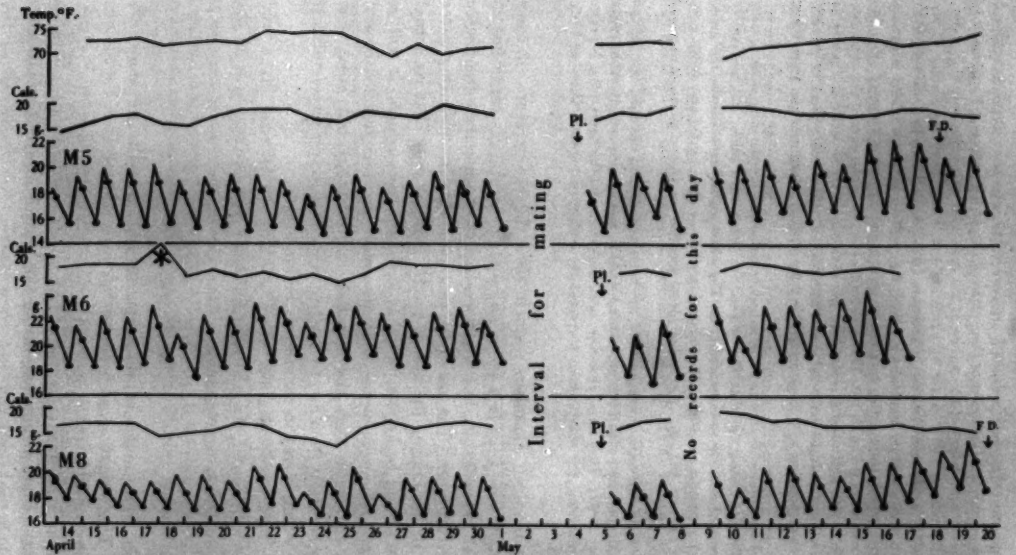

Fig. 2. From below upwards, alternately, weight curves and records of metabolic rate (in small calories per g. per hour) of mice M 8, M 6 and M 5, respectively. The top line is a record of mean room temperature in ° F. The upright marks on the abscissa represent midnight. Pl. indicates the time at which seminal plugs were found, and F.D. the time of fœtal destruction. For further details see text. The experiments were terminated on account of the mice being accidentally killed.

weight of foodstuff metabolized, so that the remaining vertical distance between the first and second circles indicates the weight of additional water excreted (plus dry weight of fæces, etc.). Above this weight curve, the metabolic rate, in small calories per g. of (high) body weight per hour of experimental period, has been plotted daily at a point on the abscissa corresponding to the midnight of each day. The mean of the maximum and minimum room temperature readings is also shown.

It is convenient to present our findings under four headings: the metabolic rate, the normal water exchange, the water exchange during pregnancy, and the loss of weight at pseudoparturition.

The metabolic rate. The major fluctuations on the graphs showing metabolic rate appear whether the calories per g. per hour are calculated from the high (evening) weights of the mice, or from the low (morning) weights. They persist even if the weight of the mouse is ignored, and the metabolism recorded as calories per hour per whole mouse. They are therefore due to uncontrolled factors which, for the following reasons, are believed to lie in the external conditions (e.g. temperature):

(*a*) The calorie curve of mouse M 4 (Fig. 3) is practically a mirror image of the temperature curve, and a trend in the same direction can be distinctly seen in many other cases.

(*b*) In the second halves of the records of mice M 5, M 6 and M 8 (Fig. 2), the temperature is relatively steady and the metabolism curves are correspondingly smooth.

(*c*) Four mice have always been under experiment simultaneously and, as a general rule, it has been observed that any marked change in oxygen consumption or respiratory quotient on one day is shared by all of them.

The following values obtained for the metabolic rates of five mice, and their variations, over periods of 25–30 days, amplify the graphical representation of the data in Figs. 2 and 3.

Mouse	Mean metabolic rate cal./g./hr.	Extreme values cal./g./hr.	Average deviation cal./g./hr.
M 3	15·5	13·5 and 18·0	1·08
M 4	14·0	12·0 „ 17·0	1·02
M 5	18·0	14·5 „ 20·0	0·92
M 6	17·5	15·0 „ 19·0	0·86 (point * neglected)
M 8	16·5	12·5 „ 19·5	0·98

The extreme values for metabolic rate were commonly found to occur when the mouse was subjected to experiment for the first time, or when there was some rapid temperature fluctuation.

In the absence of a continuous record, the effect of temperature cannot be properly assessed, particularly as the day temperature may

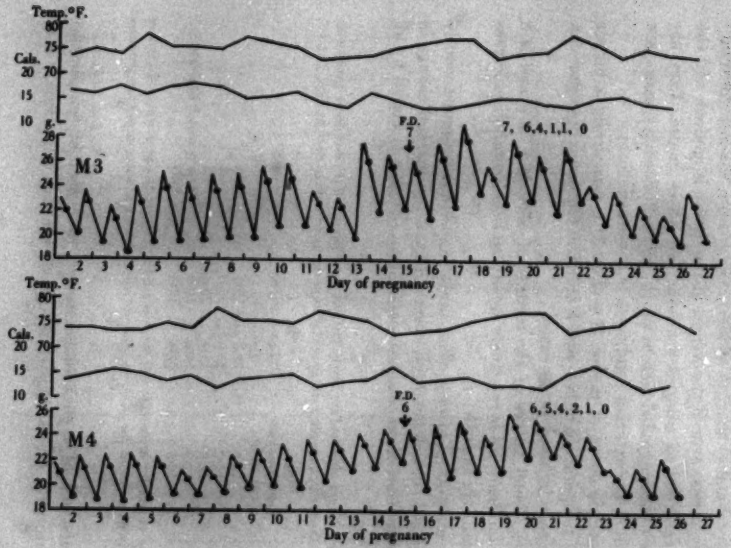

Fig. 3.

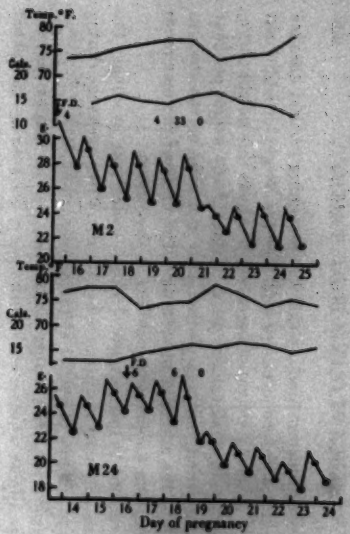

Fig. 4.

Fig. 3. Records of pregnant mice M 3 and M 4. In each case, top line shows mean room temperature, middle line metabolic rate (small calories per g. per hr.), bottom line weight. F.D. as before. The numbers over the weight curves show at F.D. the number of foetuses destroyed, and subsequently the number of placentæ found at morning and evening examinations. For further details see text.

Fig. 4. Records of pregnant mice M 2 and M 24. Arrangement and symbols as in Fig. 3.

affect the feeding of the animals. We believe that the degree of constancy of the metabolic rate could be greatly improved by rigid temperature control, and that the method might be useful for the biological assay of substances affecting metabolism.

For our present purpose, it is clear that no significant change occurs in the metabolic rate per g. body weight at the onset of pregnancy, at fœtal destruction, or at pseudoparturition (Figs. 2, 3, 4).

Mouse M 6 *asterisked point* (Fig. 2). This point illustrates a significant change in the metabolic rate. During the night the animal pulled down the soda-asbestos tray, which fell so as to confine the mouse in the lowest part of the desiccator. It was struggling to free itself when found, and had presumably been doing so during the night. We shall refer later to the subsequent day's feeding and the loss of weight on the following night.

Normal water exchange. The weight curves show that most of the diurnal fluctuation is due to the ingestion and excretion of water. The weight of water ingested was not actually measured but, since the mice showed no progressive loss of weight over a long period, they must have been in metabolic equilibrium, and the weight of water ingested must have been equal to the weight excreted. The latter quantity was measured for 17 hours of each day. It totalled, during the 18 days before mating, for M 5, 51·7 g.; for M 6, 47·5 g.; and for M 8, 28·0 g. (Fig. 2). The average daily (17 hour) excretion, calculated as a percentage of the mean average daily weight of the mice, was: for M 5, 17 p.c.; for M 6, 13 p.c.; and for M 8, 9 p.c. If we assume that the rate of excretion remained the same during the other 7 hours of the day or, at least, did not diminish, the percentages must be increased to 24, 18 and 13, respectively, to indicate the total daily exchange.

Since the mice were kept under identical conditions, no reason can be advanced for the wide difference between these averages, and it must be concluded that they express simply differences of individual habit. Occasionally the individual daily values vary considerably from the appropriate average, but no corresponding variation in any other quantity measured daily has been found. The water exchange must, therefore, be studied as it stands, and regarded as an independent quantity. The habit of each mouse is, however, characteristic, and inspection of the graphs is sufficient to indicate what order of deviation would constitute, in each case, a definite departure from the normal.

The most striking feature of the water exchange is its magnitude, the significance of which, for this investigation, will be apparent when we discuss the loss of weight at pseudoparturition. There is nothing in the experimental conditions to suggest that the high exchange is abnormal.

A few drops of water were placed in each chamber at the beginning of every experiment, and the humidity in the chambers has been shown to remain between 40 and 50 p.c. under these conditions [Newton, 1937].

The water exchange during pregnancy. Like the metabolic rate, the general character of the water exchange remains unaltered after the beginning of pregnancy, and is on the whole unaffected by fœtal destruction. The experiment recorded in Fig. 1 is sufficient to establish the general rule that the mean weight suffers little change at this time, and M 3 (Fig. 3), M 5 (Fig. 2) and M 24 (Fig. 4) show how the mean weight can remain constant and the "swing" of the weight curves remain virtually unaltered. In M 4 (Fig. 3) there is an initial tendency to loss of weight, but this is balanced by an increased gain on the next day, the weight curve resuming its previous level and appearance. The marked fall in weight of M 2 (Fig. 4), after fœtal destruction, furnishes the only exception, and must be accepted as such. It is possible that the animal was affected by being first introduced into the chamber on the night after fœtal destruction.

This steadiness in weight is surprising, for at the 15th day of pregnancy the total weight of each fœtus, with placenta, membranes and fluid, is 0·4–0·5 g., and of this less than 0·1 g. is due to the placenta. Destruction of, say, six fœtuses should therefore lead to a loss of 2·0–2·5 g. weight. This loss could be quite abrupt, for it would be made up almost entirely of water. The fœtus itself weighs about 0·15 g., and according to Moulton [1923] is at least 85 p.c. water, so that of the weight of fœtus and amniotic fluid together, nearly 95 p.c. is water. The fact that this water is not lost suddenly can mean only that some agency is actively causing it to be retained.

In the four mice which were under observation for the first 15 days of pregnancy (M 3 and M 4, Fig. 3; M 5 and M 8, Fig. 2), there were twenty fœtuses, and the total increase in mean weight of the mothers by the 15th day was 9·0 g., i.e. a gain of 0·45 g. per fœtus. This weight agrees so well with that of 15th day fœtuses (including amniotic fluid, etc.), that it is tempting to assume that the uterine contents account approximately for the increase in weight of the mothers. On this basis the changes observed could be summarized by saying that the mice, during pregnancy, put on weight which is almost entirely accounted for by water. This water is localized in the uterus, but after fœtal destruction it is retained in the body and transferred elsewhere, possibly to the tissues in general. This probably actually happens in mice subject to our metabolism experiments, but certain other facts need to be explained.

Ten records were taken at random of pregnant mice of the same strain. Their activities had been in no way curtailed and by the 15th day they had had an average gain in weight of 0·95 g. per fœtus, with a total of fifty-six fœtuses. Under normal conditions, therefore, more weight is added than can be accounted for by the uterine contents, and it is impossible to say whether or not this is also due to retained water. But whatever the magnitude of the gain in weight, it always remains or disappears as a whole (Fig. 1), and the fact that part of it is intra-uterine and part extra-uterine before fœtal destruction, and all of it extra-uterine afterwards, does not seem to influence the course of events. The presumption is that the whole weight-gain is controlled by a single factor which determines that the ingestion of water shall exceed the excretion. The balance varies in different animals, but must be sufficient to provide for the water of the fœtuses.

The loss of weight at pseudoparturition (M 3 and M 4, Fig. 3; M 2 and M 24, Fig. 4). In all cases except that of M 3 (which was subject to a rise of room temperature) pseudoparturition was accompanied by a slightly increased metabolic rate, though the increase was within the limits of experimental error. It could be sufficiently well accounted for by the increased muscular activity consequent on the delivery of the placentæ, possibly associated with a specific dynamic action following their digestion and absorption. There is no evidence of any increase in metabolism large enough to draw upon the tissues. Neither is there any sign, except in M 24, of an increase in the rate of water elimination, the water loss during the night of pseudoparturition being normal, and the next night a little less than usual. The loss in weight then is caused primarily by the failure to put on weight during the first day after delivery of the last placenta, the cumulative effect of two successive nights' normal loss being to lower the mean weight. It is important to note that in all the mice of the present series, except M 4, the excess of nightly loss over daily gain persists beyond the first day after pseudo-parturition. The loss of weight of the mice on which Fig. 1 was based also extended over more than one day. The inference from this is that the failure to gain weight during the period spent outside the metabolism chamber is due to a diminution in the volume of water drunk, and not due to an undetected polyuria. It is difficult to believe that polyuria occurs only when the animal is out of the chamber, and is followed by a somewhat subnormal rate of water loss (see weight curves) as soon as it is put back.

There are two possible causes for lack of drinking: first, a refusal of water as such; secondly, a refusal of food. A starving mouse loses weight

very rapidly, because the breakdown of body tissue for food renders superfluous a relatively large volume of water. A mouse requires roughly 1·5 g. foodstuff per day, and if it supplies this from its own tissues, its total loss of weight might theoretically amount to 4 or 5 g. [see Adolph, 1933, for a discussion of general principles]. The use of stored fat might tend to conserve weight (see Kaplan & Chaikoff, 1936], and it is true that the respiratory quotients of M 2, M 3, M 4 and M 24 on the night after delivery of the last placenta ranged between 0·70 and 0·74, whereas previously they had fluctuated round 0·8. Nevertheless, the normal non-pregnant mice, M 11 and M 12 (Fig. 5), showed a striking loss in weight when they were placed in metabolism chambers for two successive nights, and, during the intervening free period, deprived of food, but allowed free access to water. Refusal of food, therefore, cannot be excluded as a cause of the loss of weight at pseudoparturition.

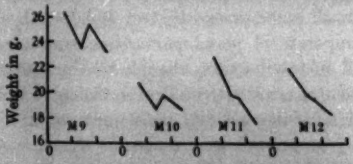

Fig. 5. Two-day weight records of mice M 9, M 10, M 11 and M 12. The marks on the abscissa represent midnight, as before, but the records are independent, the time scale in each case starting at 0. Mice M 9 and M 10 were deprived of drinking water, but allowed (moist) food, and M 11 and M 12 were deprived of food but allowed drinking water, during their periods out of the metabolism chamber.

The converse experiment was made with normal non-pregnant mice, M 9 and M 10 (Fig. 5), which were deprived of drinking water during the free period between two nights in the metabolism chamber. Their food, to which they had free access, contained 55 p.c. water. As was to be expected, their gain in weight during the day was small, but their weight loss the following night was also small. This suggests that if the water of the diet is sufficient to allow for the excretion of products of metabolism, the excess ingested is largely a matter of habit. That it varies from animal to animal has already been shown. More important is the indication that if intake (accompanied by food) is restricted, output is correspondingly restricted. Therefore, if the loss of weight in the animals after pseudoparturition is due to simple refusal of water, the succeeding night's loss, though not greater than normal, must be regarded as a *relative* polyuria.

Whether the loss of weight after pseudoparturition is due primarily to refusal of water or refusal of food, it is a positive process. On the day and night following the night of abnormal activity, mouse M 6 (*, Fig. 2) showed a similar loss of weight, but this was quickly restored. Inspection of the weight curves reveals other instances where there was a temporary fall. In normal animals there seems to be a strong tendency towards maintenance or restoration of weight and water content which is lacking in the mice after pseudoparturition.

Discussion

The precise sequence of metabolic events following pseudoparturition is of small importance compared with the interpretation of the results of the investigation as a whole. The following main points stand out. As pregnancy advances, an increase in weight of the mother takes place, becoming obvious from about the 8th to the 10th day, after which it becomes more rapid. In the animals used for this investigation, we can say that increased weight is due to the fœtuses, and that the water which it represents is localized in the uterine contents. After the amniotic sacs have been burst, and the fœtuses destroyed, the expected result would therefore be the prompt loss of this additional weight. If the placentæ are removed at the same time, this loss occurs [Newton, 1935], but if they are left in situ, the water is retained. This is in spite of the fact that, except for the placentæ, the uterus is soon empty, while the quite considerable volume of fluid often expressed per vaginam must later be made up by increased ingestion. The animal does not return to its original weight until the placentæ have been delivered, and the loss of weight at this time is accounted for to all intents and purposes by water. This seems to show that the placenta has the specific effect of giving rise to water retention. M 3 and M 4 (Fig. 3) are particularly interesting, for they were each left finally with a single placenta, and did not show the typical weight loss until this had been delivered.

One of the most certain indications of humoral activity on the part of an organ is the occurrence of positive and negative general effects coinciding respectively with its presence and absence, but only if its sole influence on the body is through the blood stream. The placenta does not quite satisfy this last criterion, for, being a temporary organ, it might by its mere presence set up nervous stimuli, the effects of which might be indistinguishable from those of temporary internal secretion. Experiments have been made recently [e.g. by Reynolds & Kaminster, 1936] showing that paraffin wax introduced into the uterus can bring about certain effects which are normally due to living contents. On the

other hand, Mohs & Guyer [1937] have shown that a carcinomatous implant into the endometrium of the rat does not inhibit the œstrous cycle as the placenta does. Histological examination of retained placentæ shows them to be living, and the same has been found in rats by Klein [1935] and Selye *et al.* [1935]. It seems probable that our results cannot be explained on the basis of the placenta acting as a foreign body.

It has long been recognized that the maternal organism accumulates water during pregnancy [see Harding, 1925; Adolph, 1933], and that the water content of the embryo during gestation is high [Moulton, 1923]. It is also well known that changes in the ductless glands occur during pregnancy, and that the anterior pituitary [Downs & Geiling, 1929], the posterior pituitary [see Heller & Smirk, 1932; Klisiecki *et al.* 1933] and the suprarenal cortex [see Harrop, 1936] can influence the water content of the body. More recently, Krohn & Zuckerman [1937] have drawn attention to the quantity of water involved in the intermenstrual œdema of the sexual skin of the macaque monkey, and from previous work by Zuckerman [1935] a direct action of œstrin seems responsible. The placenta postpones œstrus in the mouse [Newton, 1935] and rat [Klein, 1935], while D'Amour *et al.* [1936] claim to have detected œstrogenic substance in rat pregnancy urine. In view of these diverse observations, a selection of which would support almost any theory, we are unable to discuss the significance of water retention, and prefer merely to place our results on record.

Only one point needs mentioning. Although the weight changes we have observed are in the main due to water, and although there is some reason for believing that water metabolism may be modified during pregnancy, our experiments do not justify great significance being attached to the water as such. Any increase or decrease in body weight is chiefly a gain or loss of water, and if one of the functions of the placenta were to cause the body to accumulate "material" for making fœtuses, this "material" would be chiefly water under the circumstances of the present experiments. The retention of water at fœtal destruction might be secondary to the persistence of a stimulus to build tissue, whether fœtal or not, and the loss of weight at pseudoparturition might be due to a breakdown of this tissue. Our experiments, designed in the first instance to detect an increased metabolic rate or an increased loss of water, are rendered ambiguous by the peculiar way in which the weight is lost. It is noteworthy in this connexion that the metabolic rate of the animal drops considerably after pseudoparturition, though it remains proportional to the body weight.

SUMMARY AND CONCLUSIONS

1. The object of this investigation has been to explain the loss of weight which takes place in mice on the delivery of retained placentæ (pseudoparturition), when the fœtuses have been destroyed some days earlier. The metabolism of normal and pregnant mice has been studied.

2. There is no significant change in metabolic rate per gram of body weight during pregnancy, fœtal destruction or pseudoparturition.

3. In non-pregnant mice, the daily water exchange is characteristic, though not quite constant, for any one animal. From animal to animal it varies greatly and appears to depend on habit.

4. Under the present experimental conditions, the gain in weight during pregnancy can be accounted for approximately by the water of the uterine contents. On destruction of the fœtuses, this excess of water is not eliminated, and its retention cannot be explained by any local requirements.

5. The excess of water is eliminated on delivery of the retained placentæ, and we conclude that its retention in the interval between fœtal destruction and pseudoparturition is due to the presence of the placentæ.

6. In normal mice, not subject to metabolism experiments, the gain in weight during the first three-quarters of pregnancy is more than can be accounted for by the uterine contents, but it is maintained after fœtal destruction and disappears after pseudoparturition. We assume that it also represents material, possibly water, taken in under the influence of the placenta.

7. The excess of water is eliminated by a restriction of intake combined with a normal rate of excretion. The restriction of intake may be secondary to a refusal of food, and the subsequent loss of weight may include a corresponding amount of body tissue as well as water.

8. No conclusions can be drawn at present regarding the significance of the water retention. The water in itself may not be of physiological importance.

9. Certain difficulties in accepting an endocrine function for the placenta are indicated, but the balance of evidence is in favour of such a function.

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THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE SURFACE TENSION OF SOME COLLOIDAL SOLUTIONS

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During an investigation into the rate of change of surface tension (S.T.) with time in urines, normal and pathological, it was found that the addition of small amounts of acid or alkali produced very considerable changes in the curves obtained by plotting S.T. against time. A study was therefore made of these changes with varying hydrogen-ion concentrations.

An examination of the resulting curves showed minima at certain *p*H values. That these minima might be associated with isoelectric points (I.E.P.) of causative substances was at once apparent. It was decided therefore to examine simpler systems of known I.E.P. to discover whether minima of S.T. occurred at such points.

The following paper records this investigation in detail.

Materials

Since biocolloids are the main subject of investigation in this laboratory, systems of naturally occurring lyophilic colloids of well-established I.E.P. were used as far as possible.

Ovalbumin. This was prepared by the method described by Errera and Hirschberg [1933] and purified by electro-dialysis [Pauli & Weiss, 1928]. The final solution contained 0·05 p.c. of protein as shown nephelometrically.

Serum albumin. This was prepared by a similar process from human serum. The final solution contained 0·05 p.c. of serum albumin.

Serum globulin was prepared as follows. The globulins were removed from 60 ml. of human serum by semi-saturation with ammonium sulphate. The resulting precipitate was treated with 100 ml. of saturated sodium chloride solution and filtered to remove euglobulins. The filtrate

was half-saturated with ammonium sulphate and the precipitate of pseudo-globulin filtered off and dissolved in 50 ml. of 0·85 p.c. sodium chloride solution. This was dialysed through collodion and finally electrodialysed to remove all electrolytes. The resulting fluid was made up to contain 0·03 p.c. protein.

Gelatin. 0·5 g. of Coignet gold-label gelatin was warmed with 250 ml. of distilled water containing 0·1 ml. of 1*N* NaOH solution.

Casein. 0·25 g. of a sample of commercial casein was shaken with 50 ml. of distilled water containing 5 ml. of 1*N* NaOH solution at 40° C. An equivalent volume of 1*N* HCl was added to neutralize and the solution diluted to 250 ml. with distilled water and filtered giving a clear fluid containing 0·065 p.c. protein.

Ovarian cyst fluid. This was withdrawn from a non-papilliferous, multilocular ovarian cyst and was free from blood. The fluid was opalescent. 1 ml. of this was diluted with distilled water to give a solution of 0·075 p.c. of protein as shown nephelometrically.

Brilliant green. A solution of 0·1 p.c. in distilled water was prepared and filtered before use.

Experimental

The technique adopted was as follows:

20 ml. of the freshly prepared solution under test were taken, appropriate small quantities of HCl or NaOH were added from Conway microburettes, the liquid shaken and allowed to stand for 20 min. The *p*H was then measured using a Cambridge valve potentiometer in conjunction with a Morton double calomel and glass electrode system. It was found that the solutions underwent a change of not more than 0·02 unit of *p*H during the time taken for each complete experiment. It was therefore possible to avoid complicating the colloidal systems by the use of buffers. After the measurement of *p*H, 15 ml. of the liquid were taken and the rate of change of S.T. with time immediately determined by the modified maximum bubble pressure method previously described [Perryman & Selous, 1935] over a range of 0–1100 sec. As described in this earlier paper it was found in all cases that S.T. falls rapidly at first, and, as time increases, more slowly, giving an S.T.-time relationship of a logarithmic form. In the present experiments the S.T. after 30 min. was in all cases so nearly constant that the value at 1000 sec. was taken as equilibrium or "static" value. These values therefore are used for comparative purposes throughout. Surface tension-time curves were obtained for each system over the range *p*H 2–11. All experiments were carried out at room temperature.

Surface tension, dynes/cm.

Surface tension, dynes/cm.

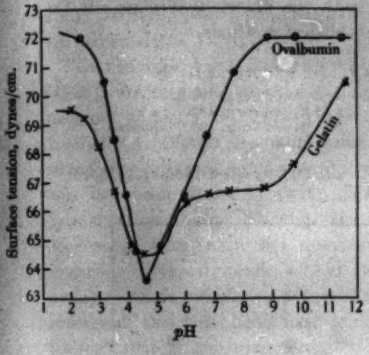


Fig. 1.

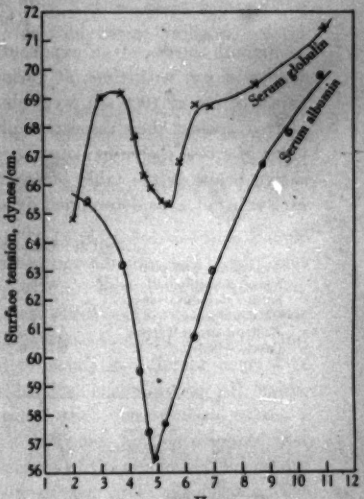
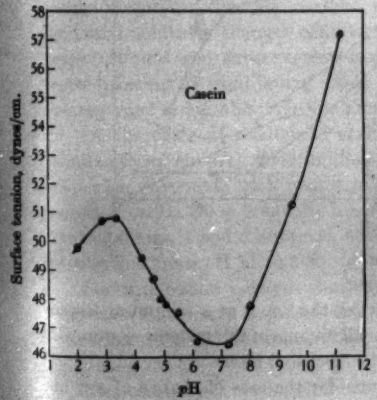


Fig. 2.



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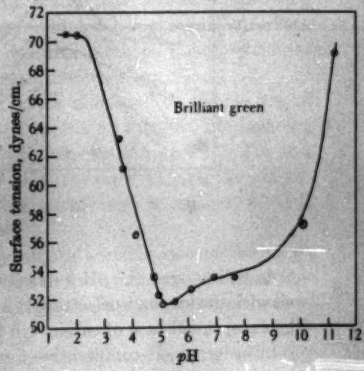

Fig. 4.

Results

Smooth curves of an exponential form were obtained showing the change of S.T. with time, at various *p*H values. For each system the values of S.T. at 1000 sec. were obtained by interpolation and these are plotted against their respective *p*H values in the figures. A minimum value of S.T. is shown in every case. The *p*H values at these minima are shown below in the table together with the known isoelectric *p*H for each substance, as determined by established methods.

	*p*H at minimum S.T.	I.E.P.
Ovalbumin 0·05 p.c.	4·55	4·55 [Svedberg, 1930]
Serum albumin 0·05 p.c.	4·85	4·88 [Svedberg, 1930]
Serum globulin 0·03 p.c.	5·4	5·5 [Svedberg, 1930] (approx.)
Gelatin 0·2 p.c.	4·75	4·7 [Limburg, 1926]
Brilliant green 0·1 p.c.	5·2–5·5	—
Casein 0·065 p.c.	6·7–6·9	4·6 [Michaelis & Pechstein, 1912]

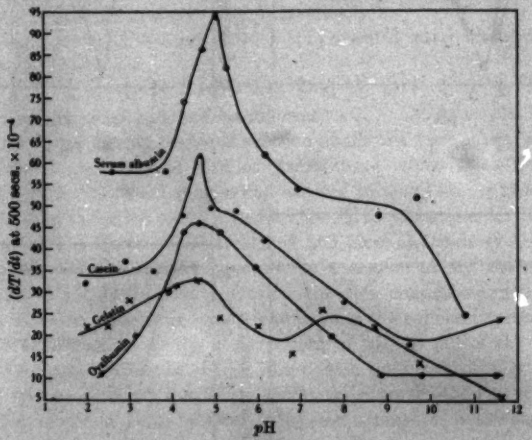

Fig. 5.

As will be seen the *p*H values, when the S.T. is at a minimum, agree well with the known isoelectric *p*H in all but one of the systems examined. The exceptional case of casein will be discussed later.

In order to get comparative figures for the rate of change of S.T. at various *p*H values the tangents to each original S.T.-time curve were

drawn at the points where $t = 500$ sec. Fig. 5 shows these values dT/dt plotted against *p*H in the cases of ovalbumin, serum albumin, gelatin and casein.

Discussion

The I.E.P. of each system examined is associated with a maximum lowering of the S.T. of the medium. This is in agreement with the observation of earlier workers [Botazzi, 1909; Buglia, 1908; D'Agostino, 1921; Johlin, 1925*a*, *b*; Loughlin, 1933; de Caro, 1925; de Caro & Laporta, 1929, 1930].

On the other hand, St Johnston [1927] and St Johnston & Peard [1926], working with solutions of gelatin, egg-albumin and casein, find maxima at the I.E.P. These contrary findings have been criticized by Loughlin [1933]. He points out that these workers allowed their solutions—adjusted to the various *p*H values—to stand for 24 hr., and this has been shown [Lewis, 1926] to result in different degrees of denaturation taking place in the solutions, according to *p*H value. Moreover, Loughlin has demonstrated that denaturation causes a lowering of the dynamic S.T. of such solutions, and so suggests that owing to these interfering effects the curves of the above workers do not represent the true behaviour of undenatured protein solutions. The present writers are in agreement with this criticism, but would further emphasize the fact that investigators who have found a maximum at the I.E.P. have used the ordinary Sugden maximum bubble-pressure, or other essentially dynamic method of measurement. The S.T. of these colloidal solutions changes considerably with time, the decrease being rapid at first and subsequently many minutes being required for the establishment of equilibrium. Such dynamic methods therefore cannot be regarded as suitable even for comparative purposes.

In the cases of ovalbumin and serum albumin the figures are of similar form, showing good minima at the I.E.P. Further, the lowering of S.T. is reversible with regard to *p*H between 2·5 and 11. For if a solution of *p*H 2·5 is gradually made alkaline to *p*H 11, and the *p*H-S.T. curve of Figs. 1 and 2 obtained, the identical curve is retraced when it is acidified from *p*H 11 to 2·5.

Serum globulin behaves similarly on the alkaline side of the I.E.P., but shows increased capillary activity at hydrogen-ion concentrations less than *p*H 3. This is conceivably due to an irreversible breakdown of the molecule.

The case of gelatin is interesting in that the minimum is definitely broad and in this agrees with Gerngross & Bach [1924] who find a

wide range for the I.E.P. of gelatin. It is well known that this substance is a complex mixture and the influence of electrolytes, from which the sample used was not freed, on the I.E.P. of gelatin may be considerable [Hardy, 1905; Wilson & Kern, 1923].

Casein shows no minimum of S.T. at the I.E.P. The protein was not specially purified, and almost certainly partly denatured. As already discussed, Loughlin [1933] has shown that denaturation may introduce appreciable masking of the minimum at the I.E.P. Moreover, it has been shown [Michaelis and von Szent-Györgyi, 1920] that in the presence of salts the I.E.P. may lie anywhere between 3·5 and 6. However, as is seen in Fig. 5, the rate of adsorption is at a maximum at the true I.E.P. of 4·6.

The case of brilliant green is noteworthy: although its properties indicate that it is not a true ampholyte and therefore cannot have an I.E.P. assigned to it, yet Fig. 4 shows a striking minimum between *p*H 5·2 and 5·5.

Other workers have found a change of S.T. with time using dilute solutions of certain dyes, but no mention is found in the literature concerning the *p*H of the solutions used, which is of paramount importance, especially as the *p*H of solutions of many dyes alter appreciably with age. Ghosh & Nath [1932] found the S.T.-time curve of a brilliant green solution of a concentration of 0·0008 g.mol. differed considerably from that of a concentration of 0·002 g.mol. and also that one of 0·004 g.mol. varied with ageing. It may well be suggested that these differences were largely due in the first case to alteration of *p*H by dilution, and in the second case to alteration of *p*H by ageing.

It is impossible to say whether a given system has a S.T.-time curve or no, unless it is sought for over a wide range of *p*H.

According to our results brilliant green has an I.E.P. between *p*H 5·2 and 5·5. Confirmation of this by other methods is unfortunately not available. It should be mentioned, however, that the solutions exhibited maximum opalescence round this same *p*H range, which indicates the existence of an I.E.P.

Ovarian cyst fluid. From the results with the simple systems examined, it seemed possible that a mixture containing several lyophilic colloids whose I.E.P. were sufficiently separated, might be analysed by examining the variation of S.T. with *p*H and identifying the constituents by means of their appropriate minima. This was accordingly done, using a dilute solution of fluid from a non-papilliferous multilocular ovarian cyst.

The resulting curve is given in Fig. 6. This shows two good minima at *p*H 5·6 and 3·85 respectively. The first of these together with the fall in S.T. at hydrogen-ion concentrations less that *p*H 3·2 are characteristic of globulin (Fig. 2). The second minimum at *p*H 3·85 has been found by us to occur in normal urines and assigned to one of the mucins which occur therein. The fluid was subsequently subjected to the usual tests, and it was found that the major proteins present were serum globulin and pseudo-mucin, thus showing the correctness of the S.T.-*p*H analysis.

In the systems examined the capillary active particles are sufficiently large to take an appreciable time for adsorption at the surface, with the production of lowering of S.T. By the method used it is possible to compare rates of change of S.T., i.e. rates of adsorption at various *p*H values. This adsorption may be conceived as a dual process of condensation and evaporation of the capillary active substance at the air-water interface, the equilibrium of these two actions at constant temperature being governed largely by the particulate size. From this point of view an attempt was made to ascertain the variation of particulate size with *p*H.

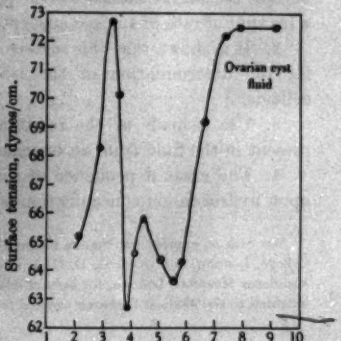

Fig. 6.

The results, as seen in Fig. 5, indicate a maximum rate of adsorption at the I.E.P. Other investigations [Hardy, 1905; Rawling & Clark, 1922; Hitchcock, 1923] have shown that protein solutions have minimum viscosity at the I.E.P. due to the association of molecules being smallest at this *p*H. Moreover, the higher degree of turbidity of such solutions at the I.E.P. has been accounted for by the particles being associated with minimal quantity of water of imbibition.

These two factors support our contention that the maximum rate of adsorption at the I.E.P. found in the experiments described is due to the particulate size being minimal at that *p*H.

Svedberg [1930], using his ultra-centrifuge technique, has found that many protein solutions show homogeneity with regard to particle weight over a wide range of *p*H including the I.E.P. Although our results support his findings with regard to stability range, homogeneity of

particle weight seems incompatible with the existence of the variations in rate of adsorption found by us. It is, however, possible that the particulate *mass* is homogeneous over a wide range of *p*H, including the I.E.P., whereas the particulate *volume* owing to stereo-chemical rearrangement and to imbibition, varies with *p*H in a manner shown by us, which variation would not effect Svedberg's sedimentation constants appreciably.

Summary

1. A number of lyophilic systems have been examined and the variation of surface tension with hydrogen-ion concentration found. Each system, with the exception of casein, shows a minimum value of surface tension at the isoelectric point: all the systems, however, show a maximum rate of surface adsorption at this *p*H.

2. It is shown that this surface tension *p*H relationship may be used for the determination of the isoelectric points of certain lyophilic colloids.

3. Use is made of the relationship to identify the major proteins present in the fluid from an ovarian cyst.

4. The great dependence of surface tension and rate of adsorption upon hydrogen-ion concentration is demonstrated and discussed.

We wish to express our thanks to Dr Phyllis M. Tookey-Kerridge, of University College, London, and to Mr G. D. Greville, of the Courtauld Institute of Biochemistry, Middlesex Hospital, London, for helpful advice in the matter of *p*H measurement. We are indebted to the Medical Research Council for a grant towards the cost of apparatus.

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SENSORY FIBRES IN THE SPINAL ACCESSORY NERVE

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(*Received* 20 *September* 1937)

Although the spinal accessory nerve has been usually regarded as a purely motor nerve, Weigner, Streeter, Fahmy, Windle, and others have shown by histological methods that it contains sensory fibres. The exact function of these fibres is not known, but Windle & De Lozier, after finding that faradic stimulation of the central end of this nerve in the neck of fourteen out of fifteen cats (twenty-five out of twenty-six nerves stimulated) produced no pain reactions, concluded that if these fibres "are capable of functioning at all they probably conduct proprioceptive impulses. Their morphology and central distribution suggest that this may be the case".

To test Windle & De Lozier's assumption the following investigation was undertaken, and in ten cats impulse discharges were recorded from the spinal accessory nerve in the neck on stretching the sternomastoid, cleidomastoid or trapezius muscles. Analysis of these discharges in single fibre preparations (eight) showed them to be similar in all respects to those arising from muscle and tendon "stretch" receptors, as found in the leg muscles of the cat by Matthews [1933].

Technique

Cats were used in all the experiments, and these were anæsthetized with dial intraperitoneally or decerebrated under ether. The spinal accessory nerve was exposed in the neck and the cat then placed in a large steam-heated box with a glass front and the finer dissection of the nerve carried out under these conditions. The technique for dissection of the nerve to obtain single fibres was the same as that first used by Adrian & Bronk [1928] and by Matthews [1933]. The cat's head was fixed firmly in a clamp to prevent movement during stretch of the muscles.

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The nerve was placed on electrodes consisting of cotton wool dipping into U-tubes of Ringer's fluid which were connected by silver wires, coated with silver chloride, to the input of the amplifying system. This was of the usual type, with recording by loud-speaker, Matthews's oscillograph, viewing screen and camera.

Results

In each experiment the spinal accessory nerve was left attached to its muscles and divided as high as possible in the neck. This was usually in the region of the ganglion nodosum, about 2·5 cm. above its entrance into the cleidomastoid muscle. The nerve was then stripped of fascia

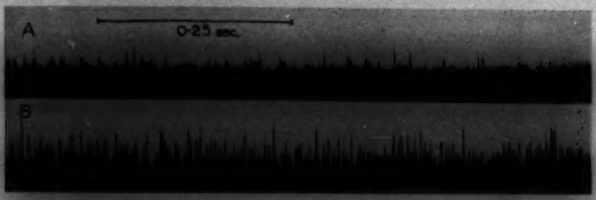

Fig. 1. Impulse discharge in the whole spinal accessory nerve. (*A*) 1 sec. after applying steady tension of 2 g. to the tendons of origin of the sternomastoid muscle, (*B*) with 20 g. stretch for 1 sec. on the same muscle.

by means of sharp dissecting needles and recording taken from its whole trunk. With the exception of the first few experiments, an impulse discharge was obtained from the nerve, in all cats examined, when the sternomastoid, cleidomastoid, or trapezius muscle was stretched. A tension of a few grams was enough to evoke an irregular discharge of impulses in many fibres and this continued as long as the tension was maintained (see Fig. 1). The trapezius muscle was not usually freed from its attachments, but light pressure over the muscle served as an adequate stimulus for an impulse discharge. That these impulses were being conducted through the whole length of nerve at our disposal (i.e. at least to the level of the ganglion nodosum) was easy to demonstrate by placing the earth electrode on the muscle as a diffuse lead and adjusting the cut end of the nerve on the other electrode so that only this cut end was elevated above the muscle, the remainder lying slack upon it. If now this elevated end was crushed the discharge ceased, but recommenced as soon as a further portion of nerve was elevated free from the surface of the muscle.

Response in single fibres

To obtain single fibre preparations the branch of the spinal accessory nerve to the sternomastoid muscle was generally used (eight preparations). By stripping away the fascia this branch was separated from the main trunk and then laid upon the electrodes. In several cases, judging from the discharge, it contained five to six sensory fibres following this procedure but in others there appeared to be at least twenty.

This branch was then dissected down until only a single fibre was functioning and the resulting discharge, on stretching the tendons of origin of the sternomastoid muscle, observed.

Fig. 2. Rhythmic response in a single fibre of the spinal accessory nerve on applying varying tensions to the tendons of the sternomastoid muscle. (*A*) 20 g. tension, (*B*) 100 g., (*C*) 200 g., (*D*) 640 g. The duration of tension before photographing was 10 sec. in each case.

The impulse discharges were characteristic of those resulting from the nerve endings in muscle and tendon as described by Matthews [1933]. A few grams' stretch on the tendon of the sternomastoid muscle was usually (six experiments) sufficient to produce them. The rate of response increased with the amount of tension (see Fig. 2), but as shown by Matthews, the maximum frequency of discharge obtained depended much more on the rapidity of the stretch than on the final degree of tension employed. In two preparations the threshold to stretch was higher (about 100 g.) and the initial frequency lower. This is probably because we were dealing with different types of "stretch" receptors [see Matthews, 1933].

Fig. 3. Rhythmic response from a single receptor, in the sternomastoid muscle, to steady tension of 200 g. on its tendons of origin. Tension applied for (*A*) 0·25 sec., (*B*) 10 sec., (*C*) 20 sec., (*D*) 60 sec. The final record (*E*) shows the response of a single receptor in the sternomastoid muscle to increasing stretch on its tendons of origin.

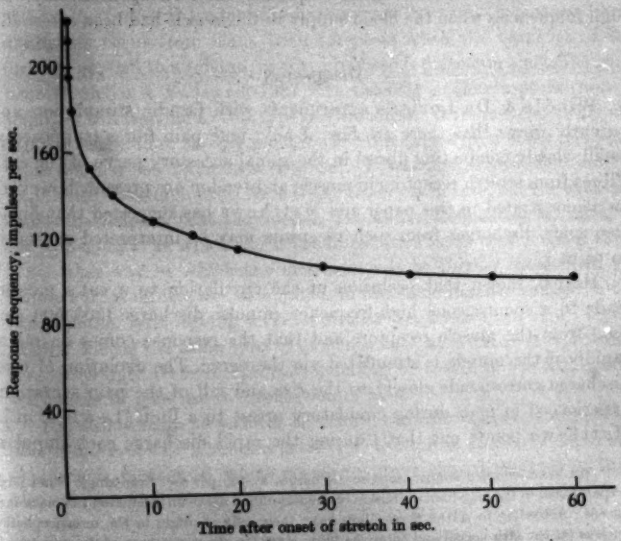

Fig. 4. Graph of response from a single receptor in the sternomastoid muscle to steady tension of 200 g. applied to its tendons of origin.

To steady tension on the tendon the endings adapted slowly (see Figs. 3 and 4). As a rule the discharge continued during the first hour at only a slightly diminishing rate and thereafter remained steady as long as the tension was maintained.

If at any time a steady tension on the muscle was suddenly reduced to a steady lower tension the discharge ceased and then gradually recommenced at a lower frequency. With very slight tension regular discharges of impulses occurred at rates as low as 10 per sec.

The highest frequency of impulses produced was 390 per sec., and this was a response to sudden stretch after the blood supply to the muscle had been cut off as completely as possible. Higher frequencies could undoubtedly have been obtained if we had employed more rapid stretch and, like Matthews, tetanized the muscle via the nerve when the circulation to the muscle had been occluded.

In only one fresh preparation was a resting discharge observed and this appeared synchronous with the heart beat. In several preparations, however, individual end organs frequently fired off spontaneously at high frequencies when the blood supply to the muscle had been arrested.[1]

Discussion

Windle & De Lozier's experiments with faradic stimulation apparently prove that there are few, if any, true pain fibres (supposedly small, slowly conducting fibres) in the spinal accessory nerve of the cat. Fibres from stretch receptors in muscle and tendon are present, however, as demonstrated in this paper and Matthews has suggested that high-frequency discharges from such receptors may be interpreted centrally as pain.

He has shown that occlusion of the circulation to a cat's muscle leads to a spontaneous high-frequency impulse discharge (300–500 per sec.) from the muscle receptors and that the response comes on more rapidly if the muscle is stimulated via the nerve. The evolution of this discharge corresponds closely to the rise and fall of the pain sensation experienced in man during circulatory arrest to a limb [Lewis *et al.*]. Matthews points out that "during the rapid discharge each impulse

[1] We have recorded similar impulse discharges at 400 per sec. from single fibres (six preparations) in the mandibular division of the trigeminal nerve on stretching the masseter muscle. According to Allen these fibres have their cells of origin in the mesencephalic nucleus (of the fifth nerve) and enter the brain stem via the motor root of the trigeminal nerve. Hence they would still be intact following sensory root section of this nerve for abolishment of pain in trigeminal neuralgia.

is set up early in the relative refractory period of its predecessor and as a result the time relations of its action current will be considerably slowed—and become more like those of the small fibres generally considered to transmit pain and hence might lead to their exciting different regions in the central nervous system and interpretation as pain instead of as muscle sense".

Although the type of stimulation (faradic) used by Windle & De Lozier would in all probability stimulate any "true" (small) pain fibres present, it seems unlikely that it was of sufficient frequency to cause a high rate of discharge in the fibres from the muscle spindles. Therefore, if Matthews's hypothesis is correct, the spinal accessory nerve might still be able to transmit pain impulses in the absence of "true" pain fibres.

Evidence to appear soon [Echlin & Fessard], however, strongly suggests that stretch receptors in muscle or tendon may discharge at or close to their maximum frequency without any sign of pain resulting. This evidence comes from experiments which show that the vibrations of a tuning fork will produce discharges from "stretch" receptors. These discharges occur from many such receptors when the branches of the fork are applied to a tendon, or when its base is placed on a neighbouring bone [Echlin & Fessard, 1937]. In the frog single receptors (muscle spindles) will respond to this type of stimulation at frequencies as high as 330 per sec., and in the cat synchronized discharges from many receptors up to 530 per sec. have been obtained. It has not as yet been possible to produce these high rates from single receptors in cats, but the evidence leaves little doubt that they occur. The above frequencies are close to the upper limit at which the nerve fibres are able to conduct and there is no other way by which the messages may be further intensified; yet if intact frogs or cats are stimulated, in the same manner as used in the experiments, with tuning forks of similar frequencies, no sign of pain results. This is the same type of evidence used by Adrian *et al.* to show that discharges from receptors responding to touch, in the frog's skin, are unable to give rise to a sensation of pain.

The experiments described, therefore, confirm Windle's suggestion that proprioceptive fibres are present in the spinal accessory nerve and evidence is mentioned which seems to prove that discharges in these fibres do not give rise to a sensation of pain.

SUMMARY

The function of the sensory fibres present in the spinal accessory nerve has been investigated by recording the action potentials in them. It has been shown that they come from stretch receptors in the muscles supplied by the nerve. These give high-frequency discharges when the muscles are stretched rapidly but it does not appear that such discharges can give rise to pain.

It is a pleasure to express our thanks to Prof. Adrian for suggesting the problem and for his help in preparing this paper: and to Dr B. H. C. Matthews and Dr D. H. Barron for their many suggestions and aid.

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OBSERVATIONS IN MAN ON A PULSE-ACCELERATING REFLEX FROM THE VOLUNTARY MUSCLES OF THE LEGS

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(*Received* 15 *November* 1937)

In a previous paper [Alam & Smirk, 1937] it was shown that a blood pressure-raising reflex may be demonstrated in normal human subjects which takes its origin from the voluntary muscles and is concerned in the rise of the general blood pressure during muscular exercise. This blood pressure-raising reflex from the voluntary muscle depends on an accumulation of metabolites in the muscle and is elicited by local muscular exercise of a forearm or a leg, provided that the rate of performance of the exercise is sufficiently great to cause such accumulation of metabolites. The reflex is elicited also when the local muscular exercise is performed at a slow rate but with the circulation through the exercising part of the limb arrested either by plunging the limb vertically into a deep bath of mercury or by inflating a sphygmomanometer cuff, placed round the proximal part of the limb, to well above the systolic blood pressure. The striking feature of this blood pressure increase, and showing clearly its reflex origin, is that (in the absence of further exercise) the blood pressure may be maintained at a high level for periods of at least half an hour by maintaining the arrest of the circulation through the exercised muscles and thus preventing the removal of the accumulated metabolites by the blood stream.

The degree of increase in the general blood pressure produced by this reflex is, under equal conditions, about the same for any given subject, but the degree of increase varies greatly from one subject to another. Thus, in normal man, the maximal increases in systolic pressure which we have been able to secure by this method have varied between 10 and 90 mm. of mercury.

In the present paper a study will be made of another reflex effect of the accumulation of the metabolites of muscular exercise in the leg muscles, namely, increase of the pulse rate.

Method

All the investigations in this paper were made on normal human subjects who were at rest in the sitting posture for some time before and throughout the experiments. The pulse-accelerating reflex was studied by observing the after effects of local muscular exercise which was performed during arrest of the blood circulation through the exercising limb. The arrest of the circulation was maintained for varying periods of time after the cessation of exercise. Pulse rates were counted immediately before exercise, during the period of arrested circulation following the cessation of exercise, and for several minutes after re-establishing the circulation through the legs.

When references are made in the text to changes in the pulse rate due to local muscular exercise, they refer to the difference between the pulse rate in the period of arrested circulation following exercise and the pulse rate at rest before the exercise. The reason for not taking as the basal pulse rate an average of the resting pulse rates before and after re-establishment of the circulation will be made clear in a future communication.

In most of the experiments the local muscular exercise was performed with the forearm or with the legs, using mainly the calf muscles. Exercise of the forearm was performed either by squeezing the rubber bulb from a sphygmomanometer at intervals usually of 1 sec., or by lifting and lowering a weight with the simple apparatus illustrated in the previous paper [Alam & Smirk, 1937]. The exercise of the legs was performed by raising and lowering the heels, thus lifting and lowering a weight of 12 kg. which was placed on the knees. An ordinary sphygmomanometer cuff was used to arrest the circulation in an arm; special large sphygmomanometer cuffs were used for the legs. The effect upon the pulse rate of local muscular exercise of other parts of the body was studied also. Exercise of the upper arm was performed by alternate flexion and extension of the arm, arrest of the circulation being secured when necessary by an inflated sphygmomanometer cuff placed as high up the arm as possible. The foot was exercised by alternate dorsi and plantar flexion of the toes, the circulation being arrested when necessary by an inflated sphygmomanometer cuff placed just above the ankle. The hand and lowest part of the forearm muscles were exercised by squeezing the rubber bulb of the sphygmomanometer, the circulation through the hand and wrist being arrested by a sphygmomanometer cuff round the wrist.

It was shown by means of plethysmographs that, in normal subjects, a sphygmomanometer cuff placed round the wrist and inflated to a pressure of 150 mm. Hg prevented the entry of blood into the soft structures of the hand, and that a pressure of 200 mm. Hg round the ankle arrested the blood flow through the soft structures of the foot. Blood flowing through bone does not concern our experiments.

In all the experiments described in this paper where exercise was performed by a limb during arrest of its circulation the exercise was continued until a moderate degree of pain developed, except in those cases where it is stated to be otherwise in the text.

Blood pressure readings were taken by the auscultatory method using mercury manometers. The low values for blood pressure at rest which are recorded in this paper are common in Egypt in healthy subjects who are capable of heavy manual labour. The three mercury manometers used in the course of this investigation were all tested against each other and against a mercury manometer in which the whole of the mercury column was visible. The readings of all manometers were identical.

Results

The effect upon the pulse rate of exercise of the legs during arrest of their blood circulation

In a series of over thirty experiments it was observed that, if exercise is performed by the calf muscles during arrest of their blood circulation, the heart rate increases. When the exercise stops but the circulation of

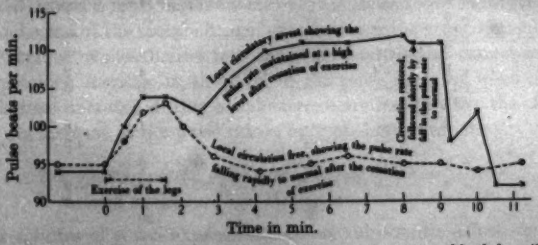

Fig. 1. A comparison of the effects upon the pulse rate of exercise of both legs (1) with arrest of the circulation through the exercising muscles, (2) with the circulation free.

blood through the exercised muscles remains arrested, the heart rate falls but not to the original normal level (Fig. 1); the heart rate then remains above the normal level for so long as the circulation is arrested and sometimes increases. This observation has been tested with periods of arrest

of the circulation through the legs lasting up to 16 min. after the cessation of exercise. The degree of increase above the normal in the pulse rate during this period of arrested circulation following exercise has ranged from 3 to 20 beats per min. (Table I). The average increase of the pulse rate was about 11 beats per min.

TABLE I. Changes in the pulse rate after local muscular exercise. The local muscular exercise was performed during arrest of the circulation to the exercising muscles. The circulatory arrest was maintained for several minutes after the cessation of exercise and the pulse rates were counted during the period of circulatory arrest and about 3 min. after the cessation of the exercise. The exercise with arrested circulation was carried in each case to the stage at which a pain of moderate severity was experienced.

	Changes in pulse rate (per min.)						
	Sites of the local muscular exercise						
Subject	Lower two-thirds of both legs	Lower two-thirds of one leg	Upper arms and forearms	Two forearms	One forearm	One foot and lower leg	One hand and wrist
A.	+12	—	−2	−10	−16	—	−10
	—	—	+4	—	—	—	−20
H.	+12	—	+9	—	− 1	—	+ 1
	+ 9	—	—	—	—	—	—
Sc.	+13	—	—	—	+ 2	—	+ 1
Sl.	+14	—	—	—	− 7	—	—
Mah.	+20	—	—	—	− 6	—	—
A.F.	+13	+1	—	—	− 6	+4	0
	+12	+5	—	—	—	—	—
Y.	+ 9	—	—	—	− 1	—	—
Moh.	+16	—	—	—	− 6	—	—
	+ 8	—	—	—	+ 1	—	—
	+ 6	—	—	—	—	—	—
	+10	—	—	—	—	—	—
	+ 9	—	—	—	—	—	—
Rah.	+ 8	+4	—	—	− 4	+2	—
	+17	—	—	—	0	0	—
	+18	—	—	—	—	—	—
Sel.	+ 3	—	—	—	− 1	−8	—
	—	—	—	—	—	+2	—
G.	+ 6	—	—	—	0	—	—
M.	+ 4	—	−3	− 6	−14	−9	—
	+ 8	—	—	—	− 5	−2	—
	—	—	—	—	−20	—	—
	—	—	—	—	− 3	—	—
S.	+10	+5	+8	—	+ 1	+2	+ 1
A.S.	+14	+8	—	− 2	− 8	−5	− 8
	—	+6	—	—	—	−1	0
	—	—	—	—	—	—	− 6
I.S.	+ 4	—	—	—	− 8	—	—
	—	—	—	—	− 3	—	—
Z.	+17	—	—	—	+ 6	—	—
A.F.D.	+12	—	—	—	0	—	—

On restoring the circulation through the exercised legs, the heart rate returns to the normal level or falls below the normal level. It is clear from a series of about thirty experiments in which arrest of the circulation has been maintained for periods of time ranging from 3 to 16 min. after the end of exercise, that the fall of the heart rate to the normal level depends upon re-establishment of the blood flow through the legs. In most experiments the fall in pulse rate to the normal on re-establishing the circulation through the legs is preceded by a short period of further acceleration of the pulse rate by a few beats per minute. This further acceleration of the pulse, when it occurs, is of about 1 or 2 min. duration in normal subjects, and is due presumably to the action of the metabolites which have been released from the legs into the general circulation. This temporary acceleration of the pulse is accompanied by hyperpnœa.

The effect upon the pulse rate of exercise of the legs without interference with their blood circulation

When the above experiments were repeated, the same amount of exercise being performed by the legs, but without obstructing the circulation through the legs, it was found in all of six experiments that the pulse rate fell back to the normal level within 1 or 2 min. after the cessation of exercise (Fig. 1). Thus the maintenance of a high pulse rate during the time while arrest of the circulation is maintained after the cessation of exercise depends upon the arrest of the circulation. It may be suggested that, even in the experiments where the circulation through the legs is arrested, exercise must involve some of the muscles above the obstructing cuff and some of the metabolites of exercise enter the general circulation. The entrance of metabolites into the general circulation cannot explain the sustained increase in the level of the pulse rate, for when all the metabolites liberated during equal exercise are free to enter the circulation, the increase of the pulse rate is only of short duration.

The effect upon the pulse rate of arrest of the circulation through the legs without the performance of exercise

In a series of seven experiments on six subjects the effect upon the pulse rate of arresting the circulation through the lower two-thirds of both legs was determined for periods of circulatory arrest lasting up to 25 min. For 10 min. before and throughout the experiments, the subjects were at rest in the sitting posture.

It was observed that, during the first 10 min. of the period of arrest of the circulation through the legs there was, with but one exception,

little change in the pulse rate. When arrest of the circulation was prolonged beyond 10 min., appreciable acceleration of the pulse rate was observed in some subjects (Table II).

Since muscle tone is regarded as a submaximal tetanus, it is not surprising that prolonged arrest of the circulation through the legs may give rise to some acceleration of the pulse and increase of the general blood pressure. It seems likely that these changes in pulse rate and blood pressure are essentially of the same nature as the larger changes in pulse rate and blood pressure which are observed after muscular exercise of a limb during arrest of its circulation.

TABLE II. The effect upon the pulse rate of arresting the circulation through the lower two-thirds of both legs without the performance of exercise.

Subject	Change in the pulse rate at the end of a stated period of circulatory arrest of the two legs		
I.S.	5 min. 0	10 min. 0	11 min. 0
I.M.	5 min. 0	10 min. −1½	20 min. −1½
A.S.	5 min. 0	10 min. 0	25 min. 1
H.	5 min. 0	10 min. 2	17 min. 9
H.	5 min. 0	10 min. 2	19 min. 6
Dr A.	5 min. 0	10 min. 0	15 min. 0
Dr S.	5 min. 3	10 min. 8	12 min. 8

In practically all the experiments on the legs set out in Table I the exercise was finished and the increase in pulse rate was established within a few minutes after the circulation through the legs was arrested. Evidently the acceleration of the pulse depends upon the muscular exercise being performed at a time when the circulation through the exercising limb is arrested.

The effect upon the pulse rate of mental processes associated with exercise

It is clear throughout our experiment that *during* the performance of muscular exercise there is an increase of the pulse rate whether the circulation through the exercising limb is free or arrested and even when the bulk of muscle involved in the exercise is small. It is generally accepted that the mental processes associated with exercise are responsible for a part of such increases in the pulse rate.

The mental effect of the exercise, however, does not explain the sustained high pulse rate which is observed when local circulatory arrest is maintained after the cessation of exercise, because the increased pulse rate persists long after exercise ceases, provided there has been no circulation of blood through the limb either during or after the exercise.

The relationship between the pain of exercise during ischæmia and acceleration of the pulse

Since exercise of the legs with their circulation arrested gives rise to pain, it may be thought that the increased pulse rate is due to the pain. It has been noticed, however, in several of these experiments that a reflex increase of pulse rate up to 6 or 8 beats per min. may be elicited by an amount of exercise of the legs which does not cause pain. For example, in the absence of pain, pulse rate increases of 8, 4, 7, 6, 5 were encountered during the period of circulatory arrest following exercise of both lower limbs in five normal subjects. Conscious appreciation of pain is not the explanation of the rise of pulse rate encountered in experiments on the legs, because even severe pain, also from exercise during ischæmia, when situated in the forearm, seldom accelerates the pulse and not infrequently is accompanied by a fall in pulse rate. Severe pain in a foot produced by exercise with ischæmia also may be associated with a fall in the pulse rate (Table I).

A comparison of the effects upon the pulse rate of exercise of different groups of muscles during arrest of their circulation

In some subjects it has been observed that, in experiments on the forearm, a fall in the pulse rate below the normal is obtained regularly during the period of circulatory arrest following exercise. In experiments on the legs, however, performed on the same subjects, the pulse rate was always increased above the normal during this period of circulatory arrest following exercise (Table I). It has been shown, however, that acceleration of the pulse is not peculiar to the experiments on the lower limbs nor is slowing of the pulse obtained only in experiments on the arms. For example, during arrest of the circulation following exercise of one foot, there was slowing of the pulse in five subjects (Table I). The same subjects showed acceleration of the pulse during arrest of the circulation following exercise of both legs. It has been shown that when local arrest of the circulation is maintained after exercise of both upper arms, the pulse is often quickened even in subjects whose pulse rates are decreased during the period of circulatory arrest following exercise of one forearm.

Our experiments would be explained if acceleration of the pulse depends upon the bulk of the muscle involved in the exercise. The pulse rates have been measured during arrest of the circulation following muscular exercise in a series of about eighty experiments (Table I). Our results comprise observations on the effects of muscular exercise with

arrested circulation in the following situations: (1) the lower two-thirds of two legs; (2) the lower two-thirds of one leg; (3) the two upper limbs; (4) two forearms; (5) one forearm; (6) one foot; (7) one hand.

As a general rule the pulse rates were greater after exercise of the larger bulks of muscle. Not all reflexes from muscles show this apparent dependence upon the bulk of muscle exercised. It has been shown that exercise of small groups of muscle in the forearms usually causes larger increases of blood pressure than exercises of the lower two-thirds of both legs. In this respect, therefore, there is a marked contrast between the blood pressure-raising reflex and the pulse-accelerating reflex, the latter being elicited more easily by exercise of both legs than by exercise of a hand or forearm.

A comparison of the effects upon the pulse rate of exercise with arrested circulation of a forearm and of two legs, the amounts of exercise being so adjusted in each pair of experiments that equal reflex increases of the blood pressure are produced

The fact that higher blood pressures are secured more frequently in experiments on the forearms than in experiments on the legs, indicates the possibility that the slower pulse rates in experiments on the arms may be due to more effective stimulation of the depressor mechanisms by the raised blood pressure. An experiment was arranged, therefore, to determine the changes in the pulse rate and blood pressure which could be secured by exercise of the two legs during arrest of their circulation. A second experiment was then performed in which exercise of one forearm with its circulation arrested was continued only until the general blood pressure was raised reflexly to the same level as in the previous experiment on the legs. The exercise of the forearm was then terminated, but the reflex increase of the blood pressure was maintained at the selected level by maintaining the arrest of the circulation through the forearm. The pulse rates which resulted from these procedures were then counted. The results of five such experiments are set out in Table III.

It is seen that when experiments are conducted in which the same increase of blood pressure is produced by exercise with obstructed circulation of either one forearm or of both legs, the pulse rate is always greater during the period of circulatory arrest which follows exercise of the legs. Therefore, one may conclude that differences in the degree of stimulation of the depressor mechanisms do not account for the differences in pulse rate between experiments on the legs and forearm.

TABLE III. The effects upon the pulse rate and blood pressure of exercise with arrested circulation of (1) a forearm, and (2) two legs. (Pulse rates are counted during the period of circulatory arrest following the exercise, not less than 2 min. after the cessation of exercise.)

	Experiments with exercise of the lower two-thirds of both legs		Experiments with exercise of a forearm	
Subject	Change in pulse rate	Change in B.P.	Change in pulse rate	Change in B.P.
A.F.	+ 5	103/47–130/74	0	100/49–132/74
H.	+11	96/60–104/71	− 1	96/58–106/67
I.M.	+ 8	84/51– 96/65	0	89/53– 98/62
	+ 6	86/53– 96/60		
S.	+13	94/60–114/80	+ 2½	89/56–110/75
M.	+ 6	106/59–144/81	−17	102/55–142/84

DISCUSSION

Our observations have shown us that when both legs are exercised during arrest of their blood circulation the pulse rate increases. The pulse rate when increased in this way is maintained at a high level *after the cessation of exercise* provided that the circulation of blood through the exercised limbs remains arrested. It has been shown that neither exercise alone nor arrest of the circulation alone can account for the increase in the pulse rate.

When the circulation through the legs is arrested, nerves form the only channel of communication between the exercised limbs and the rest of the body, and it follows that the acceleration of the pulse must be due to nerve impulses leaving the exercised limbs. The dependence of the increase of pulse rate upon exercise of the muscle, and its association with muscular pain of the intermittent claudication type indicate that the nerve impulses which are responsible for the increase of pulse rate take their origin in the voluntary muscles of the limb. The only reasonable interpretation of these results is that the accumulation of metabolites of exercise in the voluntary muscles stimulates nerve endings, and gives rise to pain of intermittent claudication type [Lewis *et al.* 1931], a reflex rise of blood pressure [Alam & Smirk, 1937], and in the case of the legs to a reflex acceleration of the pulse. The pain, blood-pressure increase and pulse acceleration all may be sustained long after exercise ceases, provided that the accumulated metabolites responsible for the pain and the reflexes are retained within the voluntary muscles by arresting the circulation through the limb.

It is of interest that the effects of exercise with locally arrested circulation of small bulks of muscle (such as the muscles of a forearm or a hand or a foot) differ from the effects of similar exercise of larger bulks

of muscle. In experiments with small bulks of muscle it was found that during arrest of the circulation following exercise, the pulse rate is diminished or unchanged even though pain is present and the blood pressure rises. Similar experiments on the legs causing equal degrees of pain or equal increases of blood pressure are associated, however, with increases of the pulse rate.

In the human subject it has not been found possible to determine the degree to which the depressor mechanisms are stimulated by the increases in blood pressure which are induced reflexly when muscles are exercised during arrest of their blood circulation. It seems likely, however, that the blood-pressure increases acting upon the depressor mechanisms tend to limit the increases in pulse rate observed in the experiments on the legs, and that in the absence of depressor reflexes the pulse-rate increases would be greater than the increases we have observed. The absence of an increase in pulse rate in the experiments with smaller bulks of muscle could be explained if it were supposed that the magnitude of the pulse-accelerating reflex depends upon the bulk of the muscle involved in the exercise. Thus the effect of the accelerating reflex would predominate in the case of larger bulks of muscle, and the effect of the depressor reflexes would be more important when only small bulks of muscle were exercised. While the above hypothesis affords a simple explanation of the results described in this paper, it is not suggested that there is evidence sufficient to justify its acceptance.

Our observations have shown that, in experiments on the legs, acceleration of the pulse and increase of the blood pressure may precede the appearance of pain. This indicates that the acceleration of the pulse and rise of the blood pressure are not dependent upon the conscious appreciation of pain. Our observations, however, do not enable us to decide whether the nerve impulses causing pain, increase of blood pressure and acceleration of the pulse are carried by the same or by different nerve fibres.

Conclusions

1. This paper is concerned with a pulse-accelerating reflex from the voluntary muscles; which reflex is considered to play a part, though a minor one, in causing the acceleration of the pulse which occurs during vigorous exercise in man.

2. The pulse-accelerating reflex is elicited when the metabolites formed during muscular exercise accumulate in a bulk of muscles such as, for example, the muscles of one or both legs. Such accumulation of the metabolites formed during muscular exercise may be secured conveniently

by exercise of a leg, the circulation of which has been arrested by means of an inflated sphygmomanometer cuff.

3. The effect of this accumulation of the metabolites is to cause discharges of nerve impulses from the leg muscles, thus causing reflexly an acceleration of the pulse, increase of the blood pressure and pain. Evidence is presented that the acceleration of the pulse often precedes the development of pain.

4. If the metabolites formed during muscular exercise are retained in the muscles by maintaining arrest of the circulation to the exercised legs after cessation of the exercise, it has been found that the pulse rate and blood pressure are held above the normal level for so long as the circulation through the limb is arrested. Equal exercise, but with the circulation free, causes only a temporary acceleration of the pulse.

5. In normal subjects, it is usually impossible to secure a reflex quickening of the pulse from a small group of muscles such as those of a forearm or of a foot even though there is severe pain of the type encountered in exercise with ischæmia. In contrast to this, reflex quickening of the pulse, during arrest of the circulation following exercise, has been obtained always after exercise of a big group of muscles such as those in the lower two-thirds of both legs. The blood pressure-raising reflex, however, does not show this apparent dependence upon the bulk of muscle involved, for usually a larger rise of blood pressure can be secured by exercise of a hand or of a forearm than by exercise of both of the lower limbs.

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THE ACTION OF CREATINE AND CREATININE ON THE CIRCULATORY SYSTEM

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The exact means by which the circulatory changes accompanying exercise are brought about, such as increased cardiac activity and local vaso-dilatation in the active muscle, have not yet been fully elucidated.

In addition to nervous mechanisms, increased attention has been drawn in recent years to the possible role of hormones (e.g. adrenaline) and of metabolites. Until 1926 the only metabolites that had been discovered which might be of importance from this point of view were lactic acid and carbon dioxide. Fleisch [1921], however, has provided evidence that the change of *p*H which they would produce is insufficient to account for the increase in blood flow through active muscle [Krogh, 1922].

The discovery of phosphocreatine and its breakdown during contraction to yield free creatine, suggested the possibility that the latter might exert a local action on the blood vessels of muscle and that some might escape into the blood stream and produce more remote effects. It has already been shown [Shapiro, 1937] that creatine has an effect on the heart of *Xenopus laevis*. The experiments to be described extend those observations to the effect on the heart of the cat and the rabbit as well as on the blood vessels of the frog. Since creatinine will arise from creatine wherever the latter is found by purely physiochemical processes [Hahn & Meyer, 1922, 1923], its effect has also been studied.

Results

Solutions of creatine and creatinine in various concentrations were prepared, using frog's or mammalian Ringer's solution according to the tissue investigated. The creatine solutions were neutral to litmus, and creatinine solutions feebly alkaline.

Action on heart

Xenopus laevis. Ringer's solution from a Mariotte bottle was perfused through the isolated heart at a pressure of 2 cm., using the Greene perfusion cannula [Sollmann & Hanzlik, 1928] tied into the inferior vena cava, fluid escaping from a divided branch of the aorta; movements were recorded from the apex of the ventricle. In some cases the Clark-Hartung method was used [Hartung, 1911; Clark, 1912].

The Ringer's solution was replaced by the solution of the drug flowing at identical pressure from another Mariotte bottle.

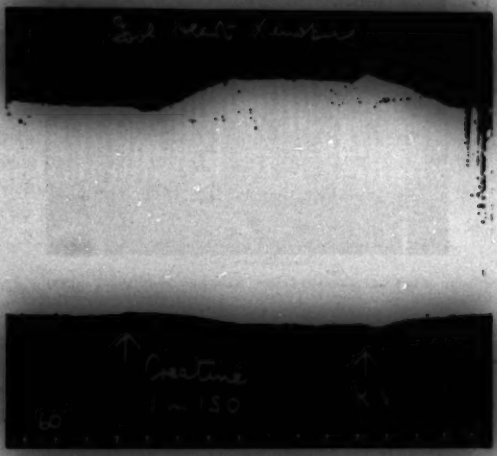

Fig. 1. Perfusion of isolated heart of *Xenopus laevis* with creatine 1 in 150. R.S. = Ringer's solution.

With creatine in concentrations of 1 in 300, or better 1 in 150, there was an increase in the amplitude of the heart beat, systole being increased by some 15–20 p.c.; the heart rate only increased slightly.

The increased amplitude of the beat was preceded by a short period during which the beats were smaller than the normal (Fig. 1); this occurred immediately after the entrance of creatine solution into the heart. These smaller beats gradually increased in size to reach an amplitude greater than normal. On the other hand, when the creatine solution was replaced by Ringer's solution a further increase in the size

of the beats occurred before they returned to the normal amplitude. The interpretation of this peculiar tracing is discussed elsewhere [Shapiro, 1937].

Creatinine produced a greater increase in amplitude than corresponding concentrations of creatine. Solutions of 1 in 500 showed improvement of the beat, systole increasing up to 100 p.c. in some experiments (Fig. 2). No preliminary decrease in the size of the beats such as occurred on perfusing with creatine was observed, and there was no further increase when the drug was replaced by Ringer's solution.

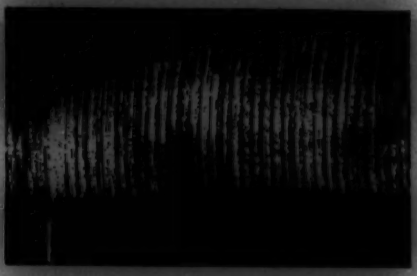

Fig. 2. Perfusion of heart of *Xenopus laevis*. At arrow creatinine 1 in 500 was introduced.

Fig. 3. Perfusion of excised cat heart. *A*, auricle; *V*, ventricle; *C*, coronary outflow. Between arrows—perfusion with 1/1000 creatinine in Ringer's solution.

Rabbit. Cat. Langendorff's method of perfusion of the coronary arteries with warm (37° C.) oxygenated Locke's solution was employed [Sollmann, 1922]. Cushny's myocardiograph was also used to record the effects on the heart *in situ*.

Creatine produced similar changes to those observed in the amphibian heart, except that the preliminary diminution in cardiac systole was not apparent. Similar results were obtained by Backman [1908] on the dog's heart.

Creatinine produced more marked increase in the size of the beat than did creatine. With solutions of 1 in 1000 a systolic increase up to 50 p.c. was obtained (Fig. 3).

Blood vessels

Perfusion of the vessels of a pithed frog. Owing to the fact that pithing is followed by a generalized loss of tone of the blood vessels this method is suited to demonstrating vaso-constrictor rather than vaso-dilator effects. It is often very difficult to produce vaso-dilation in this preparation even with such a well-known vaso-dilator drug as amyl nitrite. Despite this fact it was possible to demonstrate a vaso-dilator action with both creatine and creatinine in twelve out of fourteen experiments using concentrations of 1 in 100 to 1 in 250. The outflow of the perfusing fluid was increased by 10–20 p.c. as a rule. On one occasion an increase of 50 p.c. was obtained.

Blood pressure

Cats were anæsthetized with paraldehyde and injected intravenously with creatine or creatinine dissolved in Locke's solution. The injections were of the order of 30–40 mg. creatine or creatinine per kg. body weight. An increase in blood pressure was obtained which was not significantly greater with solutions of creatine and creatinine than with control injections of Locke's solution. The absence of any significant influence on blood pressure, despite the fact that creatine and creatinine stimulate cardiac activity, may possibly be explained on the assumption that the vaso-dilator action of creatine and creatinine, as demonstrated in the toad, also occurs in the cat and counterbalances the effect of cardiac stimulation on the blood pressure.

Conclusions

A distinct increase in the amplitude of the mammalian and amphibian heart was obtained with creatine and creatinine, as well as some evidence of vaso-dilatation, but no significant increase of blood pressure. The concentrations used were greater than would occur *in vivo*. It is, therefore, probable that creatine and creatinine do not play any important

part in the physiological adaptations of the cardiovascular system in exercise, but they may be classified, like lactic acid and carbon dioxide, as substances which can to some extent assist in producing these changes.

SUMMARY

1. Creatine and creatinine produce an increase in amplitude of the beat of the perfused isolated heart of *Xenopus laevis*, the rabbit and the cat.

2. Creatinine produces a greater increase in amplitude of the heart beat than corresponding concentrations of creatine. A 50 p.c., or greater, increase is produced by a 1 : 500 solution of creatinine in toads and by a 1 : 1000 solution in rabbits and cats.

3. Both creatine and creatinine produce a slight but definite dilatation of the blood vessels on perfusing pithed toads. They have no significant effect on blood pressure in the cat.

4. The concentration required to produce the above effects is too great to suggest that creatine and creatinine are agents of any importance in the production of the cardiovascular changes which accompany muscular exercise, but they may to some extent assist in producing these changes.

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THE ACTION OF YOUNG'S GLYCOTROPIC FACTOR OF THE ANTERIOR PITUITARY GLAND

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The work of Houssay and his collaborators has established the presence in crude extracts of the anterior pituitary gland of a hormone which plays a role in the body antagonistic to that of insulin. This work has recently been summarized by Houssay [1937]. Since the first publication considerable progress has been made towards the isolation of the individual hormones present in the original crude "diabetogenic" extract, and one characterized by its ability to suppress the hypoglycæmic effect of insulin has now been prepared in a stable form by Young [1936*a*, 1937]. Young [1936*b*] has suggested the name of glycotropic factor for

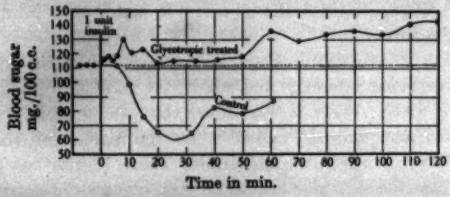

Fig. 1. An example of the effect which Young found to follow the injection of 1 unit of insulin intravenously on the blood sugar of a normal animal (circles) and a normal animal treated with the glycotropic factor (discs). Resting blood-sugar level, control 107 m.g./100 c.c., glycotropic treated animal 110 mg./100 c.c.

this substance, and has shown that the substance is distinct from prolactin and the gonadotropic and the thyrotropic factors [Young, 1937]. When animals have been treated with hypophyseal extracts containing this glycotropic factor, Young found that injection of insulin results in little or, with his later extracts, no depression of the blood sugar but rather in a subsequent hyperglycæmia (cf. Fig. 1). This striking effect has

naturally aroused much interest as to the nature of the mechanism responsible for it. Working with a less pure extract Cope & Marks [1934] obtained results which led them to suggest that a pituitary factor rendered the liver glycogen less stable so that depression of the blood sugar was rapidly countered by outpouring of glucose from the liver, often to such an extent that the blood sugar was not only restored to normal levels but was raised to abnormal heights. This view accorded well with the work of the same authors as to the sensitivity of hypophysectomized animals to insulin, for they obtained evidence that in such animals liver glycogen is abnormally resistant to mobilization and is, therefore, not readily available to oppose a depression of the blood sugar. In effect their results suggested that hypophysectomy retarded, and that anterior pituitary extract facilitated, the mechanism which normally ensures the return of the blood sugar to normal levels after injection of insulin.

Mann & Magath [1923*a*], Frank *et al.* [1924*a*, *b*] and Burn & Dale [1924] have shown conclusively that the most striking action of insulin in the body is acceleration of the rate of removal of sugar from the blood into the peripheral tissues. It is, therefore, possible that the glycotropic factor produces its effect in part by antagonizing the peripheral action of insulin, and in this case it would have claim to be regarded as a true anti-insulin. The present work was undertaken as a consequence of observations on patients with diabetes mellitus, which rendered it essential to establish whether or not the glycotropic factor thus inhibited peripheral insulin action.

In a series of investigations concerning the effect of the composition of the diet on sugar tolerance and sensitivity to insulin [Himsworth, 1933, 1934*a*, 1934*b*, 1935], it was shown that the sensitivity to insulin of both men and animals varied when the particular subject was receiving diets of different composition. These results compelled the realization that the efficiency of insulin in an organism was not constant but was governed by another factor, the sensitivity of the organism to insulin. The possibility was then raised that a type of diabetes mellitus might exist which was due, not to lack of insulin, but to the insensitivity of the individual to insulin. On investigating human diabetes, from this point of view, two types of diabetes were distinguished. In one insulin acted on the blood sugar as strongly as in healthy subjects; in the other the action of insulin was so weak that in some cases it was hardly detectable. These latter insulin-insensitive cases were further investigated and it was shown that, in them, the failure of insulin to act was due, not to masking of its action by excessive outpouring of glucose from the liver,

but to its inability to effect the removal of sugar from the blood to the peripheral tissues. In contrast, in the insulin-sensitive diabetics, insulin produced as rapid a removal of sugar from the blood to the peripheral tissues as it does in normal subjects [Himsworth, 1936]. The suggestion followed that in insulin-sensitive diabetics the disease is due to deficiency of insulin, whilst in insulin-insensitive patients diabetes mellitus results, not from lack of insulin, but from insensitivity to insulin.

In view of the work of Houssay and his school [*vide* Houssay, 1937], showing in dogs that hypophysectomy ameliorated pancreatic diabetes whilst hypophyseal extracts produced temporarily a state similar to diabetes mellitus, the idea naturally presented itself that the pituitary gland might be concerned in the pathology of insulin-insensitive patients. But there were difficulties in applying this view. All the work then available on the action of extracts of the anterior pituitary gland in antagonizing the action of insulin could be explained, on the suggestion of Cope & Marks [1934], that these extracts masked the effect of insulin upon the blood sugar by facilitating that compensatory outflow of glucose from the liver which is induced by hypoglycæmia. Nowhere was there any evidence that any extracts of the hypophysis inhibited insulin action in the peripheral tissues.

But soon after the work differentiating clinical diabetes into two types, a paper appeared by Marks [1936] on the peripheral action of a "crude prolactin" extract of the anterior pituitary gland. Using decapitated, eviscerated cats receiving a constant injection of glucose and intermittent injections of insulin, he was able to show that, in such preparations previously treated with the pituitary extract, less muscle glycogen was deposited and the blood sugar declined more slowly than in the untreated, control animals. He thus demonstrated that the pituitary extract acted in the peripheral tissues by interfering either with the disposal of glucose or with the action of insulin in accelerating this disposal. His own conclusion was in favour of the latter alternative, and recently Marks has informed us that the first possibility can be excluded on the grounds that the amount of glucose it is necessary to infuse in order to maintain constant the blood-sugar level of the decapitated eviscerated preparations, is approximately the same whether the preparations had previously been injected with pituitary extract or not.

This paper of Marks was quickly followed by the work of de Wesselow & Griffiths [1936] which demonstrated by a new test two different types of human diabetes mellitus. The basis of their differentiation was the presence in the human serum of one type of a substance which, on

injection into rabbits, produced a state of relative insensitivity to insulin. They pointed out that the insulin depression curves they obtained on the rabbits rendered insulin-insensitive by injection of serum were similar to the insulin depression curves obtained by Young [1936] on rabbits rendered insulin-insensitive by injection of the glycotropic factor. Clinically the cases whose serum antagonized the action of insulin in rabbits were of the type which were differentiated as insulin-insensitive by the test described by Himsworth [1936]. The obvious inference was that the substance which produced insensitivity to insulin in normal rabbits in a similar manner to anterior pituitary extracts, and which Marks's work indicated might also antagonize insulin in the peripheral tissues, might be the same substance which produced insensitivity to insulin in the peripheral tissues of the insulin-insensitive diabetics.

Our investigations were, therefore, undertaken to obtain evidence whether or not the glycotropic factor might act by antagonizing insulin action in the peripheral tissues of animals in which the metabolic processes had not been disturbed by anæsthesia or recent operative trauma. The scope of the experiments was later widened to determine if the glycotropic factor acted directly itself or indirectly by stimulating some other endocrine gland to activity, and to investigate the suggestion of Cope & Marks [1934] that the pituitary factor promoted excessive outpouring of sugar from the liver in response to hypoglycæmia.

General methods

The glycotropic factor. We are indebted to Dr F. G. Young, of the National Institute for Medical Research, for generous supplies of this substance. Without such co-operation the present work could not have been carried out. Details of the preparation and properties of this substance are in process of publication by Young. Here we need only say that the active substance in this extract has been found by him to be distinct from the gonadotropic, thyrotropic and lactogenic principles.

Blood-sugar estimation. The blood sugar was estimated by the Hagedorn-Jensen method. In the experiments on thyroidectomized, adrenalectomized and hypophysectomized rabbits the estimation was made on whole blood. The values thus obtained will be referred to as blood-sugar values. In the experiments on completely and partially "hepatectomized" animals the protein-free filtrate of the blood taken for the first and last samples was fermented [Somogyi, 1927], and the residual, unfermentable reducing substance estimated. The average of these two estimations was subtracted from the values given by the

Hagedorn-Jensen method on the other samples which were not fermented, and the resulting figure was regarded as the glucose content of the blood. This procedure appears permissible as the value for the unfermentable reducing substance in rabbits' blood varies little; in sixty-three separate samples on thirty-five different rabbits the value was 14·63 mg./100 c.c. (S.D. ± 5·04). For distinction the results obtained when fermentation was employed will be called blood-glucose values.

The blood samples were taken from the marginal vein of the ear, with the exception of the last sample from the "hepatectomized" animals, which died in hypoglycæmia. This was obtained by heart puncture immediately after death. Before commencing an experiment three separate samples of blood were taken for determination of the resting blood-sugar level.

Insulin. 1 unit of insulin given intravenously was used as the test dose save where otherwise stated. Crystalline insulin was used in some of the earlier experiments, but it being found that "Danish Leo" insulin gave equally reliable results, this preparation was used in the later experiments.

Preparation of animals. Male rabbits approximately 2 kg. in weight were used. At least 1 month elapsed between their coming into the animal house and the performing of the experiment. They all received a diet consisting approximately of 150 g. of a mixture of one part of oats to two parts of bran and 150 g. of fresh cabbage.

For 4 days preceding an experimental observation the animals were kept in a warm room irrespective of whether or not there had been a preceding operation. This was essential when the rabbit had been operated upon 4 days before, but those rabbits which had not been operated upon were also kept under the same conditions to ensure uniformity in the preparation for the experiment. During this period their ordinary diet was supplemented by a daily allowance of 20 g. sucrose in water.

At 6 p.m. on each of the four days before an experimental observation, the rabbit received 4 g. of glucose subcutaneously (40 c.c. of 10 p.c. solution in water), in order to ensure that the operated animals had adequate stores of carbohydrate (Fig. 5).

On the night before the experiment food was withdrawn from the cage at 6 p.m. On the morning of the experiment the animals were taken down to the laboratory at 9 a.m. and placed in front of a warm fire. Half an hour before the experiment was due to begin they were placed in a warmed box, the temperature of which was maintained by means of a

rubber hot-water bottle. They remained in this box throughout the experiment. No anæsthetic of any kind was used during, or for at least 4 days before, any experiment.

The glycotropic factor was given in two doses; one at 6 p.m. on the previous night, the other at 9.15 a.m. on the morning of the experiment. It has been found by Young that at least 3 hours are required for the action of the substance to become manifest. For this reason all experiments were commenced at 12.30 p.m.; in the pituitary-injected animals of necessity, in the controls for uniformity.

The action of the glycotropic factor in the peripheral tissues

In order to study the effect of the glycotropic factor on the action of insulin in the peripheral tissues, it is essential to eliminate the factor of hepatic glycogenolysis which, in animals treated with the glycotropic factor, is apparently enhanced to such a degree as to overcome the effects of insulin on the blood sugar. The most effective way to achieve this is to remove the liver, but this procedure cannot be recommended for the reason that observations must be commenced on the hepatectomized animals within 2 hours of operation when the effects of anæsthesia [Evans *et al.* 1931; Murphy & Young, 1932] and operative trauma [Himsworth, 1938] are still present. In rabbits which have been subjected to control operative procedures shortly before the experimental test, the glycogen stores are so depleted that the glycotropic factor may show little effect and the full effect of this factor cannot be obtained until 2 or 3 days have elapsed after an operation. The procedure, therefore, which we adopted was, not to cut the liver out of the anæsthetized animal, but to exclude the liver from the circulation in the unanæsthetized animal. The method for doing this has been described elsewhere [Himsworth, 1938]; the principle is as follows.

Two operations are performed at an interval of 3 weeks. In the first the portal vein is constricted and the tributaries entering it above the constriction ligatured. As a result a collateral circulation develops to carry the portal blood flow. At the second operation a ligature is placed round the portal vein and hepatic artery which are carrying blood to the liver and another ligature round the vessels draining the main part of the liver. The ligatures are not tied but left loosely knotted, and the ends are brought out through each lateral abdominal wall. The abdomen is closed and the animal sent back to the warm room. Four days later, when the animal is fully recovered, the liver can be painlessly and instantaneously excluded from the circulation by tightening the ligatures. By this method

90 to 94 p.c. of the liver is completely excluded from the circulation; 10 to 6 p.c. has its afferent blood supply cut off, but its efferent vessels still patent. The animals in which the liver has been excluded behave exactly like animals that have been completely hepatectomized by an acute operation, save that they are in much better condition. The advantages of the method are obvious. Trauma, fright and anæsthesia are eliminated, recovery to a normal condition is permitted, and observations can be commenced within a few seconds of eliminating the liver. It is thus possible to follow, from the moment of its inception, the spontaneous fall of blood glucose, which occurs after exclusion of the liver and to study the effect of different conditions upon an animal deprived of its liver before the preparation has been poisoned by the accumulation of those products of metabolism which are normally disposed of in that organ.

The results considered in this section concern the spontaneous decline of the blood glucose in animals in which the liver has been excluded, and the acceleration of this decline by means of insulin. For ease of comparison of individual experiments it is an advantage if the resting blood-glucose level of all the animals be at the same level. In practice such uniformity is naturally not obtained, so we have expressed all the blood-glucose values of each experiment as a percentage of the resting blood-glucose value. The justification for this procedure is provided by the work of Scott & Dotti [1932]. It will be seen that the resting blood-glucose values, being so close together (Table I), the procedure has affected the actual figures but little.

In the experiments of this section in which the glycotropic factor was given it was desired that a strong effect of this factor should be produced. The dose of glycotropic factor given was, therefore, double the amount required to efface the hypoglycæmic effect of 1 unit of insulin in the normal animal. 12 c.c. of extract, corresponding to 4 g. of fresh anterior pituitary gland, were given both at 6 p.m. on the evening before and at 9.15 a.m. on the morning of the experiment.

Results

The spontaneous decline of the blood glucose after exclusion of the liver and the acceleration of this decline after injection of insulin were investigated both in animals previously treated with glycotropic factor and in control animals which have not received this treatment.

Exclusion of the liver was effected at 0 min. This procedure occupied about 10 sec. In the experiments in which insulin was given 1 unit was injected intravenously within $\frac{3}{4}$ min. after hepatic exclusion.

TABLE I. Rabbits in which the liver was completely excluded.

	Controls				Treated with glycotropic factor			
	Spontaneous fall		Insulin		Spontaneous fall		Insulin	
No. of animals	7		5		6		7	
Wt. of animals (kg.)	2·03	(0·22)	2·04	(0·16)	1·98	(0·15)	2·06	(0·14)
Liver wt./body wt.	0·045	(0·011)	0·044	(0·003)	0·049	(0·007)	0·049	(0·009)
Time of first convulsion (min.)	278·5	(68·7)	82·0	(16·9)	178·5	(55·4)	180·0	(67·7)
Time of death (min.)	291·8	(70·0)	90·4	(14·2)	186·7	(56·6)	200·8	(67·5)
Initial blood glucose (mg./100 c.c.)	100·1	(7·00)	111·4	(12·08)	107·2	(12·72)	114·1	(15·64)
Blood glucose at death (mg./100 c.c.)	19·5	(3·86)	27·0	(7·40)	19·5	(4·46)	18·6	(9·69)

The figures in brackets show the standard deviation (S.D.).

TABLE II. Rabbits in which the liver was completely excluded

	Controls				Treated with glycotropic factor			
	Spontaneous fall		Insulin		Spontaneous fall		Insulin	
Time min.	Fall of blood glucose p.c.	S.D.	Fall of blood glucose p.c.	S.D.	Fall of blood glucose p.c.	S.D.	Fall of blood glucose p.c.	S.D.
0	100·0	0·00	100·0	0·00	100·0	0·00	100·0	0·00
5	89·0	1·63	87·6	4·22	91·2	4·06	87·7	4·56
10	83·0	4·66	75·8	5·08	82·8	3·02	82·7	4·49
15	79·6	5·92	64·6	5·24	81·2	3·06	75·7	3·95
20	75·4	7·61	57·8	5·43	74·2	6·57	71·1	4·16
30	76·4	7·07	48·0	4·94	66·3	8·06	57·9	6·13
40	71·9	4·61	37·6	8·66	60·3	6·50	51·6	7·50
50	64·6	8·73	34·4	7·96	52·3	7·32	46·4	6·65
60	59·0	7·72	30·8	5·72	50·0	9·02	43·9	7·26
70	53·6	9·02	27·8	5·67	45·2	7·82	43·1	11·02
80	52·7	7·63			43·2	10·38	44·8	11·44
90	51·1	8·44			40·8	10·76	42·3	13·37
105	50·9	6·15			34·5	11·31	38·0	15·76
120	50·9	10·08			32·3	8·64	42·0	10·27
135	43·4	8·94			34·5	7·30	37·2	13·15
150	40·9	7·87			32·0	9·27		
160	36·7	8·20						
180	35·1	7·45						
200	31·7	4·59						
220	27·7	7·54						

Time refers to minutes after hepatectomy. The figures for each set of animals are terminated when such a number of animals had died as to make the average figures of no significance.

S.D. = standard deviation.

The results are summarized in Tables I and II. It will be seen from Table II that, in each group of experiments, the standard deviation (S.D.) of the percentage blood-glucose values at a definite time after exclusion of the liver is small and, therefore, the curves drawn through

the mean values of the blood glucose allow a fair comparison to be made between the different sets of experiments. In Figs. 2, 3 and 4 the mean value curves are compared.

In Table I are given data showing that the animals in the four different groups of experiments were comparable. Their weights were approximately the same and the ratio liver weight/body weight determined after death also agrees within close limits. This ratio is a good index of the skill with which the operative procedures, necessary before exclusion of the liver, have been carried out [Himsworth, 1938]. The initial

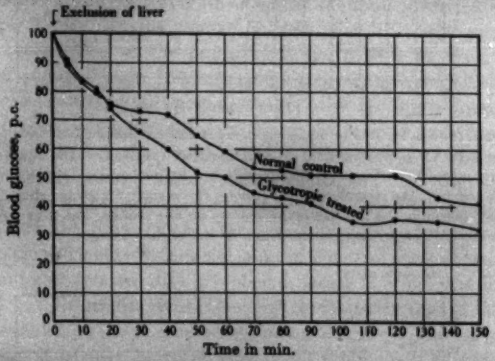

Fig. 2. The spontaneous fall of the blood glucose, expressed as a percentage of the resting value, (1) after complete exclusion of the liver in rabbits previously treated with the glycotropic factor (half discs), and (2) in the control series of rabbits not so treated (discs). The curve for the glycotropic treated rabbits is the average for six animals; the curve for the normal control rabbits is the average for seven animals. The liver was excluded at 0 min.

blood-glucose values in the four groups vary relatively little, but on the whole the initial blood-glucose level is higher in those animals which have received the glycotropic factor. The average initial value for all twelve control animals is 104·8 mg./100 c.c. (S.D. ± 11·29), whilst for all thirteen animals treated with the glycotropic factor it is 110·9 mg./100 c.c. (S.D. ± 14·78). Young [1937], using unoperated rabbits, obtained the same difference; 104 mg. blood sugar/100 c.c. for the controls in comparison with 111 mg. blood sugar/100 c.c. for the animals which had received the glycotropic factor.

In Fig. 2 the spontaneous decline of the blood glucose after exclusion of the liver in the control rabbits and in the rabbits previously given the

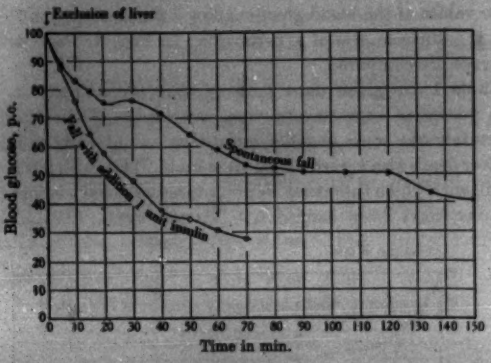

Fig. 3. Comparison of the rate of fall of the blood glucose, expressed as a percentage of the resting value, in control rabbits, (1) after complete exclusion of the liver (discs), and (2) after complete exclusion of the liver followed by intravenous injection of 1 unit of insulin (circles). The spontaneous fall curve is the average for seven rabbits, the curve in which insulin is also injected is the average for five rabbits. The liver was excluded at 0 min., the insulin was injected within 45 sec. of the exclusion.

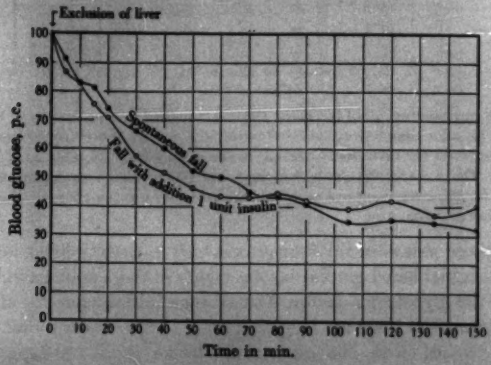

Fig. 4. Comparison of the rate of fall of the blood sugar, expressed as a percentage of the resting value, in rabbits previously treated with the glycotropic factor, (1) after complete exclusion of the liver (discs), and (2) after complete exclusion of the liver followed by intravenous injection of 1 unit of insulin (circles). The spontaneous fall curve is the average of six rabbits, the curve in which insulin is also injected is the average of seven rabbits. The liver was excluded at 0 min., the insulin was injected within 45 sec. of the exclusion.

glycotropic factor are compared. The curve for the rabbits treated with glycotropic factor falls slightly faster than for the control animals. Table I shows that the former animals also convulsed and died in hypoglycæmia considerably earlier than did the controls. The blood-glucose values at death, however, were the same, 19·50 mg./100 c.c. The possible significance of the difference in rate of decline is not clear, but the results do show definitely that the glycotropic factor does not retard the spontaneous disposal of glucose by the peripheral tissues.

Figs. 3 and 4 show the effect of 1 unit of insulin on the rate of fall of blood glucose in the control animals and in the rabbits treated with the glycotropic factor. In the control animals (Fig. 3) insulin produces a marked acceleration of the fall so that the rabbits receiving insulin convulsed and died some 200 min. earlier than those in which the blood glucose was allowed to fall spontaneously (Table I). It is of interest that the terminal blood-glucose values in the insulin-treated controls averages 8 mg./100 c.c. higher than in any of the other groups of experimental animals. In the animals treated with glycotropic factor (Fig. 4) insulin produces little if any acceleration of the rate at which the blood glucose declines, and the times of the first convulsions and of death, and the terminal blood-glucose values are almost identical with those for the control animals treated with glycotropic factor in which the liver was excluded but no insulin given. The slightly increased rate of fall in the first hour shown by the insulin-treated animals is, in our opinion, probably significant. It is clear, however, that in the rabbits receiving the glycotropic factor, the action of insulin in accelerating the rate of removal of glucose from the blood by the peripheral tissues is practically eliminated.

In order to eliminate the possibility that the effect of the glycotropic factor in antagonizing the peripheral action of insulin might be a non-specific effect of foreign proteins, rabbits prepared for exclusion of the liver were injected with cold saline extracts of rat muscle or rat liver or with human plasma. These injections were given in the same proportions and at the same times as the injections of glycotropic factor. The liver was later excluded and 1 unit of insulin given intravenously. In every case (9 animals) the fall of blood glucose after insulin occurred at the same rate as in the insulin-treated control rabbits which had received no previous injections. The antagonization of insulin by the glycotropic factor thus appears to be specific.

It may, therefore, be concluded that the action of insulin in accelerating the rate at which the peripheral tissues remove blood glucose is antagonized by the glycotropic factor. The rate of spontaneous removal

of blood glucose by these tissues is, however, not diminished by this factor.

The role of the liver

In animals that have been previously treated with the glycotropic factor, the blood glucose declines in the absence of the liver. The liver is, therefore, as important for the maintenance of the resting blood-sugar level in such animals as it is in the normal controls. In rabbits previously injected with the glycotropic factor, deprived of the liver and given insulin, the blood sugar always declines until death occurs in hypoglycæmia. In control animals which have been prepared for exclusion of the liver, treated with the glycotropic factor, but in which the liver is not

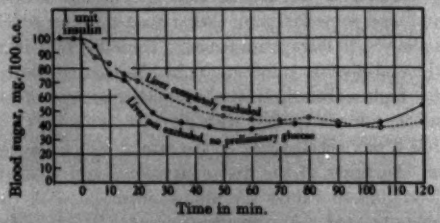

Fig. 5. Curve (discs) showing that, after preliminary treatment with the glycotropic factor, the action of insulin in depressing the blood sugar is not abolished unless the animal has previously received adequate supplies of carbohydrate. For comparison the average curve showing the fall of blood sugar for glycotropic treated animals in which the liver has been completely excluded at 0 min. and 1 unit of insulin injected intravenously immediately, is given.

excluded, injection of insulin produces either no change in the blood-sugar level or a hyperglycæmia (Fig. 7). The liver is, therefore, essential for that maintenance of the resting blood-sugar level and for the production of those hyperglycæmias after injection of insulin into animals treated with the glycotropic factor.

The question which now arises is: from what substance does the liver derive the glucose which it pours into the blood to produce these hyperglycæmias? This glucose may be formed from glycogen or from non-carbohydrate sources. The following experiment suggests that the source is liver glycogen.

It is well known that trauma [Claude Bernard, 1877] and anæsthesia [Evans *et al.* 1931; Murphy & Young, 1932] reduce the glycogen content of the liver. If an animal is prepared for exclusion of the liver but, after the last operation, the injections of 4 g. of glucose are not given

each evening, it will be found that such an animal, although treated with the glycotropic factor, shows a perfectly ordinary depression of the blood sugar following insulin injection even though the liver is not excluded. It can be seen from Fig. 5 that the insulin depression curve under these circumstances is practically the same as the curve obtained, after injection of insulin, on animals similarly treated with the glycotropic factor but in which the liver has been excluded. In these experiments there was neither undue trauma nor excessive anæsthesia at the preliminary operations. If the glycotropic factor acts by stimulating rapid new formation of sugar from non-carbohydrate sources, there is no reason why it should not have produced the usual antagonization of insulin in these preparations. The preparations differ only from those of Fig. 7 in that they had not received preliminary glucose and it, therefore, appears reasonable to suggest that hyperglycæmia follows insulin injections into rabbits treated with the glycotropic factor only when the liver is well stocked with glycogen.

The question as to what stimulus provokes the liver of animals previously injected with the glycotropic factor to secrete sugar into the blood at such excessive rates, after injection of insulin, must next be considered. If we accept the evidence offered by Cope & Marks [1934] that the liver glycogen in these animals is unstable and unusually easy of mobilization, there appear to be three main possibilities. First, the stimulus may be the excitement consequent upon the intravenous injection; secondly, it may be the direct result of insulin upon the liver; and thirdly, it may be fall in blood-sugar level. The first possibility is disproved by the fact that in these animals hyperglycæmia does not follow intravenous injection of normal saline; the second possibility has not been examined because there is good evidence in favour of the third.

The effect of fall in the blood-sugar level was investigated on partially hepatectomized animals. If 90 p.c. of the liver is excluded in the rabbit, the blood sugar sinks until death occurs in hypoglycæmia. The animals were prepared as for total exclusion of the liver but, at the second operation, the ligatures were so arranged as to leave 10 p.c. of the liver still in the circulation. The post-operative preparation of these animals, both in the control rabbits and in those treated with the glycotropic factor, was exactly the same as detailed in the previous section of this paper. Doubt may be felt as to whether the 10 p.c. of the liver remaining in the circulation after the ligatures were drawn tight might survive in a healthy condition. Frank & Isaacs [1910] have shown that the first result of liver damage

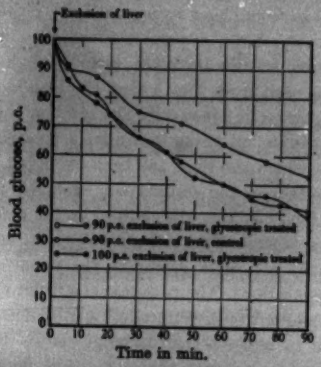
Exclusion of liver
Blood glucose, p.c.
90 p.c. exclusion of liver, glycotropic treated
90 p.c. exclusion of liver, control
100 p.c. exclusion of liver, glycotropic treated
Time in min.

Fig. 6. Curves showing the spontaneous fall of the blood sugar (1) after exclusion of 90 p.c. of the liver in rabbits previously treated with the glycotropic factor (circles) (average of five animals); (2) after exclusion of 90 p.c. of the liver in control rabbits not receiving this treatment (discs) (average of six animals); (3) after total exclusion of the liver in rabbits previously treated with glycotropic extract (half discs) (average of six animals). The liver was excluded at 0 min.

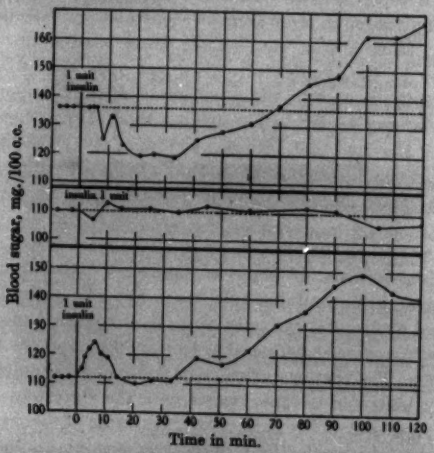

Fig. 7. Diagram showing the different types of curve that may follow the intravenous injection of 1 unit of insulin into rabbits previously treated with the glycotropic factor.

is an "explosive discharge" of liver glycogen. We have found as much as 1·8 p.c. of glycogen in the 10 p.c. of liver left in the circulation.

Two groups of rabbits prepared in this manner were used to study the rate of spontaneous decline of the blood sugar after partial exclusion of the liver. One group only received the glycotropic factor. In Fig. 6 the average curves for the spontaneous decline of the blood sugar in these two groups are shown and compared with the average spontaneous decline curve for rabbits treated with the glycotropic factor and in whom the whole liver was excluded. It will be seen that the spontaneous decline curve for the rabbits, treated with glycotropic factor, and still retaining 10 p.c. of the liver, falls more slowly than does the curve either for the control animals with partial exclusion, or the animals treated with glycotropic factor with total exclusion of the liver. This result shows that after injection of the glycotropic factor the output of sugar from the liver, in response to a fall of blood sugar, is greatly increased so that, from the inception of the spontaneous fall of blood sugar, glucose is poured into the blood from the remaining 10 p.c. of liver in amounts sufficient to prevent the blood sugar falling as rapidly as it does in the control animals.

In Fig. 7 are shown the various types of blood-sugar curve following the intravenous injection of 1 unit of insulin into rabbits treated with the glycotropic factor. These animals were exactly comparable with the rabbits used for investigation of the peripheral action of the glycotropic factor. They had been prepared for exclusion of the liver but the ligatures were not tightened, and the liver was thus kept in the circulation. It will be seen that there are three types of curve. In one the blood-sugar level remains unaltered; in the second the blood sugar drops a few mg. and then rises above the original level; in the third the blood sugar first rises, next falls slightly and then rises again. These curves suggest that if the blood-sugar level is not disturbed by the injection of insulin no hyperglycæmia results, but if the level is disturbed an outpouring of sugar into the blood occurs and hyperglycæmia follows. As the liver is necessary for the production of such hyperglycæmia, then the change in blood-sugar level presumably acts by stimulating the liver to an increased output of sugar. It has been shown in the experiments on the partially hepatectomized animal treated with the glycotropic factor, that the spontaneous fall of blood sugar is antagonized from its onset. It appears then that the smallest fall of blood sugar is sufficient, in animals so treated, to set in rapid motion the mechanism of glycogenolysis in the liver. It is thus easy to understand how hyperglycæmia may follow in the curve

characterized by a small initial depression of blood sugar. In the case of the curve commencing with an initial hyperglycæmia, and in which a sustained hyperglycæmia follows even though the blood sugar at no time sinks below the resting level, an artefact is probably involved. The intravenous injection of commercial insulin, into normal rabbits, is followed immediately by a transient hyperglycæmia. With purer insulin the hyperglycæmia is hardly noticeable, but if such an insulin is injected into animals treated with the glycotropic factor, a well-marked initial hyperglycæmia is often obtained. This hyperglycæmia is probably due to a trace of impurity in the particular preparation of insulin. It is to this that we attribute the initial hyperglycæmia in our curves, and we attribute the subsequent continued hyperglycæmia to the stimulation resulting from the small drop in blood sugar after this initial rise.

From a consideration of the results presented in this and the previous section the following explanation is suggested. The liver is not directly concerned in the antagonization of insulin action by the glycotropic factor. This antagonization occurs primarily in the peripheral tissues. If it is complete no disturbance of blood-sugar level results; as a consequence the liver is not stimulated to pour sugar into the blood, and the blood-sugar level remains steady. If, however, the antagonization is incomplete, the insulin which escapes inhibition accelerates the disposal of sugar in the peripheral tissue and a fall of blood sugar results. As after the injection of the glycotropic factor the liver reacts to fall of blood sugar by an excessive outpouring of sugar into the blood, a fall in blood sugar, however small, immediately sets the mechanism of glycogenolysis in motion to such an excessive degree that the blood is flooded with sugar. Accelerated removal at the periphery being retarded by inhibition of insulin action to a greater or less degree, this sugar accumulates in the blood and hyperglycæmia results.

The role of the thyroid

Cope & Marks [1934] have shown that injection of crude saline extract of the anterior pituitary gland retards or prevents the depression of the blood sugar after injection of insulin both in normal rabbits and in thyroidectomized rabbits. It seemed, therefore, desirable to see if the glycotropic factor, which had been separated from other hormones contained in their crude saline extract, would antagonize the effect of insulin in thyroidectomized rabbits.

The rabbits were thyroidectomized 4 days before the test. In these 4 days they received exactly the same preparations as did the other

animals used in this investigation. 6 c.c. of a preparation of the glycotropic factor, which had been obtained from 2 g. of fresh anterior pituitary gland, were injected subcutaneously at 6 p.m. on the evening before and at 9.15 a.m. on the morning of the test. Three hours later 1 unit of insulin was given intravenously and the depression curve measured. One week after this experiment a control insulin depression curve was carried out on the same animal when it had received the routine preparation for experimental observation but had not received the glycotropic factor. These two experiments were carried out on each of seven thyroidectomized rabbits with exactly the same results. The animals were killed after the second experiment, and serial sections of the site of operation made. In each case the thyroid had been removed completely.

In Fig. 8 are shown the results obtained on one animal, and these prove that the thyroid gland is not necessary for the action of the glycotropic factor.

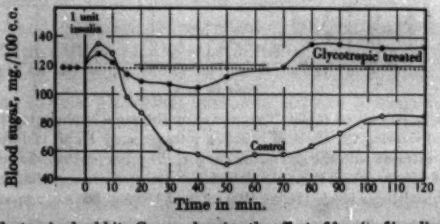

Fig. 8. Thyroidectomized rabbit. Curves showing the effect of 1 unit of insulin intravenously on the same thyroidectomized rabbit after it had previously been treated with the glycotropic factor (discs), and when it had received no such treatment (circles). Resting blood-sugar levels 119 mg./100 c.c. and 118 mg./100 c.c. respectively.

The role of the pituitary gland

In view of the fact that the glycotropic factor represents but one of the hormones of the anterior pituitary gland which are concerned with carbohydrate metabolism [Young, 1936*b*] it is important to know if the glycotropic factor can act in the absence of this gland. If it can then it acts directly; if it cannot, then presumably one of the other pituitary hormones is required to act in synergism with it.

Shortly after we had commenced the investigation of these possibilities we learnt that Dr F. G. Young was also investigating the same point in greater detail. As our results were in agreement we shall only record our main finding and leave the details to be reported by Dr Young.

Hypophysectomy was performed 6 months to 1 year before these experimental operations were carried out. During the 4 days before the test the animals received the routine preparation, and each of the two doses of the glycotropic factor corresponded to 2 g. of fresh anterior pituitary gland. 1 unit of insulin was given intravenously in the test after treatment with the glycotropic factor, but in the control test only ½ unit of insulin was used as, owing to the increased susceptibility of hypophysectomized animals to insulin, larger doses produce dangerous and often fatal hypoglycæmia.

Serial sections of the sella turcica and base of the brain revealed in each of our animals some remnants of pituitary tissue in the region of the tuber cinerium. In the three animals we used for this investigation the

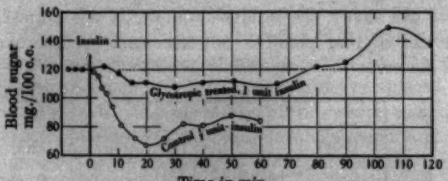

Fig. 9. Hypophysectomized rabbit. Two curves on the same hyphophysectomized rabbit, one (discs) showing the effect of injecting intravenously 1 unit of insulin after preliminary treatment with the glycotropic factor, the other (circles) showing the effect of ½ unit of insulin when the animal had not received such preliminary treatment. Resting blood-sugar levels 120 mg./100 c.c. and 116 mg./100 c.c. respectively.

remnants amounted only to two or three islets of a dozen or so cells which were surrounded by scar tissue. It is probable that these were of little significance, as the rabbits showed marked evidence of absence of the pituitary hormones by atrophy of the testes, suprarenals and liver [Himsworth & Scott, 1938].

The results of the two experiments on such an animal are shown in Fig. 9. It will be seen that, in the practically complete absence of the pituitary gland, the glycotropic factor still antagonizes the action of insulin.

The role of the adrenal glands

Considerable disagreement exists as to the role of the adrenal glands in carbohydrate metabolism, and especially with regard to the relationship between this gland and the hypophysis. Viale [1933] reported that pancreatic diabetes in the dog is ameliorated after adrenalectomy, and

Houssay & Biassotti [1936] obtained the same result in toads. Gondard [1933], Lewis & Turcatti [1933] could not confirm this. Using depancreatized cats, however, Long & Lukens [1936] have consistently obtained results showing that, after complete removal of the adrenal glands, the survival time of these animals is greatly prolonged although not to the same extent as after hypophysectomy. Similar discrepancies are found in the reports on the effect of "diabetogenic" extracts of the anterior pituitary gland on adrenalectomized animals. Houssay & Leloir [1935] state that adrenalectomy does not reduce a hyperglycæmia previously produced by such extracts in dogs, and further Houssay & Biassotti [1936] found that implantation of the pituitary gland into adrenalectomized toads still causes hyperglycæmia. On the other hand Long [1936–7] finds that in adrenalectomized partially depancreatized rats "diabetogenic" extracts are without effect. Conflicting as these results are they give the impression that, in some species at least, there exists between the pituitary and adrenal glands an interrelationship which influences carbohydrate metabolism. We, therefore, determined to investigate the effect of the glycotropic factor of the anterior pituitary gland on the reaction of adrenalectomized rabbits to insulin.

The right adrenal gland was first removed and 3 weeks later the left adrenal gland was taken out. The experiment was performed 4 days after the second operation. In these 4 days the animals were given by subcutaneous injection 0·72 g. of sodium chloride night and morning and 4 g. of glucose at night. They did not receive cortical hormone, as we wished to avoid any possible complicating factors. That the administration of sodium chloride kept them in good condition during this period is indicated by the fasting blood-sugar level on the fourth day which, in the seven rabbits of this series, averaged 113·5 mg./100 c.c. (S.D. $\pm$ 6·8). Total adrenalectomy was performed on two other animals, but these were discarded because they were in bad condition and their fasting blood-sugar values were below 100 mg./100 c.c. The dose of glycotropic factor given was the same as before, namely two injections each corresponding to 2 g. of fresh anterior pituitary tissue. 1 unit of insulin was used as the test dose.

We were fortunate enough to keep three of these seven animals in good condition for another four days after the first experimental observation and in these we carried out a control insulin depression curve when they had not received the glycotropic factor. It is of interest that three of the remaining four animals died during the night following the

first test. This was surprising, because they were in excellent condition at the end of the test.

All the animals were allowed to live as long as possible, 20 days being the maximum, in order to allow time for any remnants of adrenal tissue to hypertrophy. After death a careful post-mortem examination was made, serial sections of the inferior vena cava and surrounding tissues from the level of the renal veins to the entrance of the first hepatic vein were cut, and sections were taken of any tissue, wherever it was found, which might have been an accessory adrenal gland. In one case only was a minute remnant of cortical tissue found.

The results in a typical experiment are shown in Fig. 10. It will be seen that, in adrenalectomized rabbits, the glycotropic factor still manifests its effect of antagonizing the action of insulin, and it can be concluded that, in rabbits, neither the adrenal cortex nor the adrenal medulla is essential to the action of the glycotropic factor.

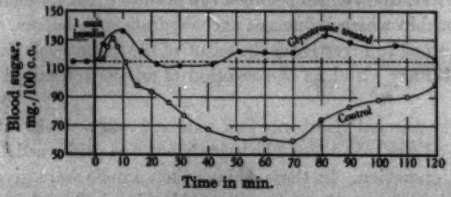

Fig. 10. Adrenalectomized rabbit. Two curves on the same totally adrenalectomized rabbit after intravenous injection of 1 unit of insulin, the one (discs) after the animal had previously been treated with the glycotropic factor, the other (circles) when it had not received such preliminary treatment. Resting blood-sugar levels 116 mg./100 c.c. and 112 mg./100 c.c. respectively.

The results obtained on thyroidectomized, on hypophysectomized and on adrenalectomized rabbits show clearly that the presence of neither the thyroid, nor the hypophysis nor the adrenal glands is essential for the action of the glycotropic factor in antagonizing the effect of insulin. It is possible that one of the other endocrine glands may be essential for this action, but as yet no definite evidence has been produced to indicate that the gonads, pineal or thymus are significantly concerned in carbohydrate metabolism. The suggestion is therefore made that, in rabbits, the effects observed after injection of the glycotropic factor are due to the direct action of that factor itself and do not require intermediation by any other hormone.

Discussion

The results obtained on rabbits which are presented in this paper can be conveniently summarized as follows. In the peripheral tissues the glycotropic factor antagonizes the action of insulin, but does not affect the rate at which the tissues utilize glucose spontaneously; the glycotropic factor influences the liver so that it responds to a decrease in the blood sugar by an excessive outpouring of glucose; neither the thyroid, nor the pituitary, nor the adrenal glands are necessary for the action of the glycotropic factor. No further discussion of the results obtained on the thyroidectomized, hypophysectomized or adrenalectomized animals is required, but the results on the animals in which the liver has been excluded partially or completely need further consideration.

The experiments on the animals in which the liver has been completely excluded from the circulation demonstrate that the glycotropic factor antagonizes the action of insulin in accelerating the rate of removal of glucose in the peripheral tissues, but does not retard the rate at which these tissues dispose of glucose spontaneously.

In considering these results we would emphasize that we are concerned with the peripheral tissues as a whole, and that, although there is little evidence that insulin affects to any extent the carbohydrate content of the peripheral tissues other than muscle, there is no justification for interpreting our results as showing that the antagonization of the effect of insulin by the glycotropic factor is of the nature of an inhibition of insulin's action in increasing the glycogen content of the muscles. We have observed one fact which justifies our stressing this obvious point. In rabbits in which the liver is excluded, the decline of blood sugar does not occur smoothly but is interrupted by a series of waves [Himsworth, 1938]. It is difficult to see how these waves can be interpreted as meaning anything other than the liberation of glucose into the blood from some hitherto unknown store of carbohydrate in the periphery. If the glycotropic factor facilitates the mobilization of glucose from this store, as Cope & Marks [1934] suggest that it does from the liver, then, in animals so treated, the action of insulin might well be masked by an inflow of glucose from the periphery, whilst in animals not treated with glycotropic factor the peripheral carbohydrate would be less easily mobilized and the action of insulin would be manifest. The work of Marks [1936] supports our doubt that such a peripheral liberation of stored glucose plays an important part in the antagonization of insulin

action by the glycotropic factor. His demonstration that, after administration of insulin and glucose to animals treated with an extract of the anterior pituitary gland similar to the one we used, less muscle glycogen was deposited than in the animals not so treated, suggests a true inhibition of peripheral insulin action in the muscles.

If we accept our results as indicating that insulin action in the peripheral tissues is actually prevented, we are brought face to face with an interesting possibility. If insulin action is inhibited then, because the rate of spontaneous fall of the blood sugar after exclusion of the liver is not faster in control animals than in the animals which have received the glycotropic factor (Fig. 1), it must be allowed that the spontaneous fall of blood sugar occurs through the agency of a mechanism which does not require the aid of insulin. That such a mechanism exists is indicated by the work of Mann & Magath [1923*b*], which shows that, after hepatectomy, the blood sugar in depancreatized dogs declines rapidly to hypoglycæmic levels. A second explanation which raises the same question is that the glycotropic factor may act by preventing the absorption of insulin, in which case, if the spontaneous fall of blood sugar after hepatectomy is brought about by a mechanism requiring insulin, the rate of fall should be faster in the control animals as compared with those treated with the glycotropic factor for 18 hours previously. Experiment shows that this is not so, and the possibility of a mechanism, which does not require insulin, for spontaneous utilization of glucose is again raised. The value of these considerations lies in the suggestion that if the spontaneous utilization of glucose by the peripheral tissues is independent of but facilitated by insulin, then the glycotropic factor must act, not by retarding such a utilization, but by interfering with the action of insulin in facilitating it. It is possible that direct experiments with isolated tissues might yield evidence supporting such a suggestion.

On comparing the curve of spontaneous decline of the blood glucose in hepatectomized rabbits which have received the glycotropic factor with control animals which have not received such treatment, it will be seen the decline in the former is more rapid than in the latter (Fig. 2). We have no explanation to offer for this. The result itself conflicts with the statement of Campos *et al.* [1933] that implantation of the pituitary gland into hepatectomized toads retards the fall of blood sugar and also of muscle glycogen. The latter finding is the opposite of what one would expect from our results and those of Marks [1936], but it should be remembered that whilst they used whole pituitary gland we used a fractionated extract of this tissue.

In the animals in which the liver had been completely excluded, the blood-glucose value at death with hypoglycæmic symptoms averaged 19·50 mg./100 c.c. after the spontaneous decline in both the glycotropic factor-treated animals and in the controls, 18·57 mg./100 c.c. in the glycotropic factor-treated animals which had received insulin, but 27·00 mg./100 c.c. in the animals which received insulin but no glycotropic factor. In other words death with hypoglycæmic symptoms occurred at a significantly higher level of blood glucose when the blood glucose fell rapidly than when it fell slowly. This accords with the clinical observation that the blood sugar may, if it falls slowly, fall to very low levels indeed, without inducing hypoglycæmic symptoms, but that if the blood sugar falls rapidly hypoglycæmic symptoms appears at much higher levels. In agreement with these observations there is also the observation that, if the rebound of the blood sugar after depression with insulin is very rapid, hypoglycæmic symptoms may persist when the blood sugar has again swung back to normal levels [Himsworth, 1933]. These results appear to be best explained by suggesting that there is a definite lag in the transference of glucose from the blood to the tissues, so that, on the one hand, when the glucose in the tissues falls rapidly, as after injection of insulin, glucose does not have time to leave the blood sufficiently rapidly to keep the blood and tissue sugars in equilibrium, whilst on the other hand when the fall is slow, transference is sufficiently rapid for the two to remain in equilibrium. If this supposition is correct, then the glycotropic factor cannot interfere with the actual transference of glucose to the tissues, for if it did the insulin treated animals with the liver excluded which had received insulin and had been treated with the glycotropic factor would have died at least as rapidly as the animals injected with insulin which had not been treated with the glycotropic factor, and further their terminal blood-glucose values would have been very high indeed.

At the commencement of this paper it was stated that this present experimental work was undertaken to test the hypothesis that insulin-insensitive cases of diabetes mellitus might be explained on the basis of the proved peripheral inhibition of insulin action in them being due to a substance similar to the glycotropic factor. The results of Marks [1936] and those reported in this paper establish that the pituitary glycotropic factor does inhibit insulin action in the peripheral tissue, and so give support to the above hypothesis. Further evidence in the same direction is provided by experiments we have recently published [Himsworth & Scott, 1938]. These show that hypophysectomized rabbits, unlike

normal rabbits, do not become insensitive to insulin when dietary carbohydrate is restricted, but that such animals when given the glycotropic factor do develop an insensitivity to insulin similar to that of normal rabbits when given a diet low in carbohydrates. The suggestion follows that low carbohydrate diets induce, whilst high carbohydrate diets restrain the secretion by the pituitary of a substance similar to the glycotropic factor. As a purely clinical observation, it has already been reported that diabetics distinguished by Himsworth's test as insulin-sensitive do not require more insulin when allowed an increased amount of carbohydrate in the diet, whilst in those patients revealed as insulin-insensitive, increase of dietary carbohydrate necessitates an increase in the dosage of insulin. It appears that insulin-sensitive diabetics react to increase of dietary carbohydrate like normal individuals by increasing their sensitivity to insulin whilst insulin-insensitive diabetics after the same change in diet, fail to increase their insulin sensitivity. Exact experimental data corroborating this impression have been obtained on diabetics and will shortly be published. If the insulin-insensitive type of diabetes is due to pathological oversecretion of a glycotropic-like substance by the pituitary gland, then it is not surprising that in these patients the physiological stimulus to restraint of this secretion, namely an increased consumption of carbohydrate, is ineffective.

Summary

1. In rabbits in which the liver has been completely excluded from the circulation, the pituitary glycotropic factor inhibits the action of insulin in accelerating the removal of blood glucose by the peripheral tissues, but it does not affect the rate at which these tissues use glucose spontaneously.

2. The liver is essential for the production of hyperglycæmia after injection of insulin into rabbits treated with the glycotropic factor. This hyperglycæmia only occurs if injection of insulin disturbs the blood-sugar level. Experiments on rabbits in which the liver is only partially excluded suggest that animals which have received the glycotropic factor react to a fall in blood sugar by an excessive liberation of sugar from the liver into the blood.

3. Neither the thyroid, nor the hypophysis, nor the adrenal glands are necessary, in rabbits, for the action of the glycotropic factor in antagonizing the effects of insulin.

4. The significance of these findings in normal carbohydrate metabolism and in diabetes mellitus is discussed.

We wish to express our thanks to Miss F. N. David of University College, London, for helping in the statistical analysis of our results and to express our indebtedness to Dr F. G. Young of the National Institute for Medical Research, not only for generous supplies of the glycotropic factor, but for many stimulating criticisms.

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THE EFFECT OF SALT DEFICIENCY IN MAN ON THE VOLUME OF THE EXTRACELLULAR FLUIDS, AND ON THE COMPOSITION OF SWEAT, SALIVA, GASTRIC JUICE AND CEREBROSPINAL FLUID

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The experimental production of salt deficiency in normal persons provided an opportunity of studying its effect upon the volume and composition of certain of the body fluids, and it was thought that it would be profitable to do so since very little information is available on the subject. Such literature as there is will be discussed in the appropriate sections of the paper. The salt deficiency was produced by diet and sweating [McCance, 1936 *a*, *b*]. The fluid intake was liberal and unrestricted. The chemical methods employed have previously been given [McCance & Widdowson, 1937*a*]. The changes in the serum and red blood cells have already been described [McCance, 1937].

Results

The volume of the extracellular fluids

Lavietes *et al.* [1936] have described methods for the determination of the volume of the extracellular fluids in man, depending upon the administration of substances which in the main do not penetrate into the cells and whose concentration may readily be measured in the plasma and urine. Sucrose was one of these substances. The method employed was to inject a known amount intravenously, and to determine the proportion of this excreted and the concentration in the plasma after stated intervals. From the amount excreted it was possible to calculate the amount left in the body at the given times and, assuming the concentration of sucrose in the other extracellular fluids to be the same as it was in the plasma, it was possible to calculate the volume of liquid in

which the amount left in the body was dissolved, i.e. the volume of the extracellular fluids. The volume of extracellular fluids can also be obtained from the change of concentration in the serum within any one period and the amount excreted in that period.

In the present experiments sucrose was injected into one subject on four occasions for kidney function tests, and inulin was injected into two others for a similar purpose [McCance & Widdowson, 1937*b*]. From the behaviour of sucrose and our knowledge of the chemical and physiological properties of sucrose and inulin, it may be assumed that the distribution of inulin in the body is, like sucrose, probably confined to the extracellular fluids. McCance & Widdowson [1937*b*] kept no record of the exact amount of sucrose or inulin injected, but the following data were available: (*a*) a record of the amount of sucrose or inulin excreted in each of four to eight successive periods of 20–40 min., (*b*) a record of the concentration of the sucrose or inulin in the plasma half-way through each period. Three methods therefore could be used for the determination of the volume of the body fluids in which the sucrose or inulin was dissolved (the extracellular fluids): (1) The total amount excreted in each period could be divided by the fall in plasma concentration within that period. The latter could be obtained graphically from the plasma concentrations determined in the middle of each period. This gave a series of results for any one experiment which could be inspected and averaged. (2) The total amount excreted during the whole experiment could be divided by the total fall in plasma concentration within that time. This is really a variation of the first method, but in practice does not always give the same answer as the averaged result obtained by it, because in each method a different experimental error tends to preponderate. (3) If it was assumed that the volume of fluid in which the inulin or sucrose was dissolved remained constant from the first period to the last, the amount left in the body (z) at the beginning of the first period could be calculated thus:

$$\frac{(z-a)\times 100}{b} = \frac{(z-a')\times 100}{b'},$$

where a and a' are the amounts excreted between the beginning of the first period and the end of any two periods and b and b' the plasma inulin or sucrose at the end of these periods. (In practice the first and last periods were used.) Hence the amount of fluid in which the inulin or sucrose was dissolved at the end of each period could be found from the concentration in the plasma and the amount left in the body at that time. All three methods have been used. The first two do not involve the

assumption that the volume of fluid in which the sucrose or inulin was dissolved remained constant throughout the experiment. This assumption, however, can be justified by the experience of Lavietes *et al.* [1936] and, so far as D.W. and J.T.B. were concerned, by the direct results of method (1). For one of the inulin subjects (R.M.L.), on whom four experiments were carried out, the first method of calculation gave results which suggested that the volume of fluid in which the inulin was dissolved increased throughout each experiment so that it was greater at the end than it had been at the beginning. This may possibly have been the case, but the same apparent result might have been obtained in the presence of a constant volume of extracellular fluid if the plasma had not been in equilibrium with the whole of it when the blood samples were withdrawn. Inulin diffuses so much more slowly than sucrose [Bunim *et al.* 1937] that something of this sort may have been happening in this particular subject. Alternatively the results may have been due to experimental variations inherent in method (1) and this appears equally probable since this method always tended to give erratic results from one period to the next. Owing to this uncertainty, it has been decided only to give and to discuss in detail the results obtained on subjects D.W. (sucrose) and J.T.B. (inulin), in both of whom the data by all three methods were consistent and satisfactory. It may be added, however, in passing that the results obtained on R.M.L. entirely support the conclusions to be drawn from D.W. and J.T.B.

The full data for one experiment on D.W. and one on J.T.B. are given in Table I and the results of all six experiments on these two men

Table I. Data from two experiments

	D.W. Second normal experiment			J.T.B. Salt-deficient experiment		
No. of period	Duration of period min.	Sucrose excreted in each period g.	Plasma sucrose in the middle of each period mg./100 c.c.	Duration of period min.	Inulin excreted in each period g.	Plasma inulin in the middle of each period mg./100 c.c.
1	30	6·5	183	19	4·5	201
2	31	5·1	142	30	4·1	124
3	30	3·7	118	30	2·7	82
4	29	2·9	83	30	1·9	55
5	26	1·9	72	42	1·8	35

are given in Table II. Both subjects were of similar build and height. As might have been expected from what is known of sodium chloride metabolism the deficiency brought about a considerable reduction in the extracellular fluid volume [Harrop, 1936], and it is interesting to note

TABLE II. A comparison of the extracellular fluid volumes during health and during salt deficiency

Subject and substance used	Method of calculation	Volume of the extracellular fluids (litres)			
		Normal (before)	During salt deficiency		Normal (after)
			1	2	
D.W. (sucrose)	1	13·5	10·3	9·4	13·4
	2	13·0	10·0	9·0	13·5
	3	14·5	10·2	9·7	13·1
J.T.B. (inulin)	1	12·4	7·5	—	—
	2	11·4	7·2	—	—
	3	12·3	7·6	—	—

TABLE III. A comparison of the loss of body weight and of the extracellular fluid volumes brought about by salt deficiency

Subject	Body weight kg.		Extracellular fluid litres		Extracellular fluids as p.c. of body weight	
	At start	Loss	At start	Loss	At start	During deficiency
D.W.	76	4·0	13·7	3·9	18	13·6
J.T.B.	76·5*	3·3	12·0	4·6	15·7	10·1

* In a previous paper [McCance & Widdowson, 1937*b*] J.T.B.'s weight at one stage of his salt deficiency was erroneously given as 87 kg. increasing overnight to 87·86 kg. The figures should have been 72·3 and 73·16 kg.

the return to the higher figure with recovery. Table III shows the extracellular fluid losses compared with the losses of body weights. In compiling this table D.W.'s preliminary findings were taken to be the normal ones and the values obtained in the two deficiency experiments were averaged and subtracted from them. These figures for the normal extracellular fluid volumes are of the same order as those obtained by Lavietes *et al.* [1936], but they suggest that inulin may tend to give slightly lower values than sucrose. This is being investigated. The recorded loss of body weight in these experiments is presumably the mean of (*a*) the loss of extracellular water, (*b*) possibly some loss of cellular nitrogen and water [McCance, 1936*a*, *b*], (*c*) a gain in cellular water due to the reduction of the plasma osmotic pressure [McCance, 1937]. Nitrogen balances were not recorded in these subjects and it is doubtful if the fluid losses have been measured with sufficient accuracy usefully to compare the expected with the actual losses of weight. It is, however, obvious that one effect of salt deficiency has been to reduce very materially the percentage of the total body weight occupied by the extracellular fluids.

Knowing the change in the volume of the extracellular fluids and the change in concentration of plasma ions [McCance, 1937] brought about

by salt deficiency, the total loss of these ions from the extracellular fluids can be calculated. Thus D.W.'s normal 13·7 l. of extracellular fluid containing 101 m.eq. of Cl/l. were reduced by salt deficiency to 9·8 l. containing 83 m.eq./l. This represents a loss of some 41 p.c. of his extracellular chloride. Similar calculations show that J.T.B. lost 51 p.c. of his extracellular chlorides. It is thought by some at the present time that, except for the red blood cells, the chloride ion is confined to the extracellular fluids. If this is so, the data now presented would give a measure of the total body chloride and the percentage loss brought about by salt deficiency. Direct determinations of chloride, however, made on whole animals suggest either that there must be a considerable quantity of chloride outside the extracellular fluids or else that the volume of the extracellular fluids as determined by sucrose and inulin is too low [McCance, 1936*a*; Lavietes *et al.* 1935, 1936; Harrison *et al.* 1936]. These substances may, however, legitimately be employed (as in the present instance) to make comparative measurements of the volume of the same man's extracellular fluids at two different times.

The composition of resting saliva

40–50 c.c. of juice were collected in a measuring cylinder without any gustatory or mechanical stimulation and sampled without being filtered. The juice appeared to be secreted at a normal rate during salt deficiency. The chlorides were determined directly, the sodium and potassium after dry ashing and the results are shown in Table IV. It will

TABLE IV. Composition of saliva

	Normal health mg./100 c.c.			During salt deficiency mg./100 c.c.		
Subject	Sodium	Chloride	Potassium	Sodium	Chloride	Potassium
J.T.B.	13	51	76	—	46	78
D.W.	22	31	77	12	46	99
R.A.M.	13	60	75	8	60	96
W.M.G.	12	36	86	9	52	135
R.M.L.	18	79	116	7	68	148
Average	15·6	51	86	9	54	111
Average (as m.eq./l.)	6·8	14·4	22	3·9	15·2	28·5

be observed that salt deficiency consistently produced a fall in the concentration of sodium and a rise in the concentration of potassium. The changes in chloride concentration were inconsistent. There is no previous work with which to compare these findings. The normal concentrations of sodium and potassium in the present investigation are on the whole lower than those of Brown & Klotz [1937]. The difference

may be due to the fact that Brown's subjects stimulated their salivary secretion by chewing paraffin. The concentrations of chloride agree with those found by Welin & Frisk [1936].

Gastric juice

It has been known for some time that the continuous removal of gastric juice from animals does not radically alter the composition of the juice [Dragstedt & Ellis, 1930]. Lim & Ni [1925–6] for instance found that the gastric glands of dogs continued to secrete in spite of dehydration and a loss of up to half the body chlorides. Katsch & Mellinghoff [1933] and Mellinghoff & Heuschert [1934] removed gastric juice from patients for 10 hr. a day for 3–5 days and found no consistent reduction in the volume or in the chloride secreted. The response to histamine was as good or almost as good on the last day of treatment as it had been on the first.

In the present investigation fasting gastric juice was removed and then 0·5 mg. of histamine hydrochloride was administered subcutaneously. Gastric juice was then withdrawn (usually at 15 min. intervals) over a period of 30–60 min. The procedure was repeated when the salt deficiency was most severe. The normal and deficient fasting juice was sometimes very small in amount and it was seldom possible to make a complete analysis so the results will not be discussed. In Table V are given the

Table V. The effect of salt deficiency on the composition of histamine-stimulated gastric juice

Subject	Vol. collected (c.c.) / Collection time (min.)	Free HCl m.eq./l.	Total HCl m.eq./l.	Total Cl m.eq./l.	Total Na m.eq./l.	Total K m.eq./l.
		Composition during normal health				
R.A.M.	99/60	45	60	106	10	—
W.M.G.	53/45	54	71	93	16·3	17·5
J.T.B.	154/45	45	66	100	20	18·5
D.W.	70/60	0	8	63	59	15·9
R.B.N.	192/60	74	94	—	—	—
P.M.E.	72/30	—	118	116	—	—
		Composition during salt deficiency				
R.A.M.	111/60	42	60	99	6·5	—
W.M.G.	65/45	56	78	104	14·1	21·2
J.T.B.	64/45	17	38	62	11·3	22·6
D.W.	92/60	0	3	57	47·5	23·6
R.B.N.	123/60	52	68	—	—	—
P.M.E.	39/30	—	105	106	—	—

results which were obtained from studies of the histamine juice. Filtered juice was used for the determination of free and total HCl, unfiltered juice for the determination of the other ions. Chlorides were estimated directly and the juice was dried and incinerated for the determination

of sodium and potassium. It will be observed that in some respects the subjects showed considerable individual variation. W.M.G. could not be made nearly so deficient as the others, and his free HCl, total HCl and chloride actually showed a small increase during his deficiency. J.T.B. on the contrary showed considerable reductions in all these constituents. It may be concluded that salt deficiency tends to reduce the concentration of acid and chloride in gastric juice but that individuals vary widely in their response. There seems to be no close relation between the changes observed in the gastric juice and those in the plasma [McCance, 1937]. Peters's [1935] review of the literature suggests that the osmotic pressure of the gastric juice should mirror accurately changes in osmotic pressure of the serum and that the concentration of chloride in gastric juice should approximately equal the concentration of base in the serum. In the present experiments the gastric juice was not withdrawn at the same time nor was it necessarily withdrawn on the same day as the blood. Further, a certain amount of saliva was swallowed, but all the same some of the findings are difficult to reconcile with Peters's generalizations. Thus the total chlorides are much too low, particularly those of J.T.B. (during deficiency) and of D.W. These low chlorides may be due to an admixture of mucus with the acid juice [Bolton & Goodhart, 1931; Welin & Frisk, 1936]. However that may be, the present experiments suggest that the mixed stomach juices may often be hypotonic. Salt deficiency appeared to bring about a fall in the concentration of sodium in the gastric juice and a rise in the concentration of potassium. It will be recalled that these changes were also noted in resting saliva.

Cerebrospinal fluid

Lumbar punctures were carried out on D.W. and R.M.L. at the time when they were most salt deficient. Blood was taken a few minutes beforehand, and the comparative results are shown in Table VI. In all

TABLE VI. Composition of cerebrospinal fluid during salt deficiency

	Sodium			Chloride		
Subject	Serum m.eq./l.	C.S.F. m.eq./l.	C.S.F. Na / Serum Na	Plasma m.eq./l.	C.S.F. m.eq./l.	Plasma Cl / C.S.F. Cl
D.W.	130	137	1·05	74	109	0·68
R.M.L.	128	130	1·03	75	109	0·69

analytical procedures cerebrospinal fluid (C.S.F.) was treated in the same way as serum [McCance, 1937]. C.S.F. was not withdrawn when the subjects were in normal health, but the concentration of the sodium and

chloride found in these subjects' normal sera has already been published [McCance, 1937], and the normal C.S.F. chloride may be taken to be 122–126 m.eq./l. (in clinical parlance 715–740 mg. of NaCl/100 c.c.). The ratios of C.S.F. sodium/serum sodium (Table VI) are of the same order as those found by Dailey [1931] in patients with normal serum electrolytes, and this, taken with the known fall in serum sodium, strongly suggests that salt deficiency has brought about a fall in the C.S.F. sodium, and that the osmotic pressure of the C.S.F. has probably fallen to about the same extent as that of the serum. The chlorides in the C.S.F. have undoubtedly fallen. Clinicians will appreciate the abnormality of these salt deficient C.S.F.'s better when it is stated that the concentration of chlorides in them amounted only to 640 mg./100 c.c. (as NaCl). There is one further point of interest. Although the sodium ratios are of the order found by Dailey [1931], the chloride ratios (see Table VI) are lower in both cases. This means that the concentration of chlorides in the C.S.F. has not fallen so much as that of the chlorides in the plasma (compare with this the behaviour of chlorides in the saliva) and is quite in keeping with the modern view that the C.S.F. is a secretion rather than an ultrafiltrate [McCance, 1936*b*].

Sweat

The sodium, potassium, chloride and nitrogen were determined in all sweats. In the earlier experiments sweat and washings were evaporated down at a faintly acid reaction and made up to a standard volume, usually 2 l. Later, the total volume of the sweat and washings was measured and samples taken for analysis without evaporation. The water lost in the sweat was assessed from the loss of body weight without correcting for the water lost in the expired air. Most subjects sweated every day for 7–10 days, but R.A.M. only sweated five times in 10 days. The routine was always the same. About 1 hr. after a light lunch the subject undressed, weighed himself and entered the previously heated radiant-heat bath. The open end was then closed with mackintosh sheeting so that only the subject's head projected, and his temperature was taken at frequent intervals and maintained as far as possible between 100 and 101° F. by altering either the ventilation or the heating. Each subject drank the same volume of water at about the same rate each day in the hot-air bath. Some subjects took 800 c.c. and others 1000 c.c. After 2 hr. the heat was switched off and 10 min. later the subject emerged and was washed down with distilled water, dried and weighed again [McCance, 1936*a*, *b*]. Conditions therefore were comparable from day

to day. As the salt deficiency became worse the amount of water lost remained about the same, varying to some extent with the subject (Table VII). The amount of sodium and chloride lost in the sweat invariably decreased, but more so in some subjects than others [McCance,

TABLE VII. The effect of salt deficiency and repeated exposure to high temperatures on the composition of the sweat

Subject and no. of sweat		Water c.c.	Sodium m.eq.	Potassium m.eq.	Chloride m.eq.	Nitrogen mg.
Total amounts excreted in "standard" sweats during progressive salt deficiency						
D.W.	1	3010	180	15·0	180	760
	2	2710	163	14·6	178	780
	3	2570	150	17·3	150	820
	4	2820	86	18·0	90	880
	5	2350	83	18·2	75	970
	6	2820	97	22·0	95	1010
	7	2480	57	19·6	54	1200
	8	2430	63	26·2	72	1300
R.A.M.	1	1700	143	7·4	120	500
	2	1810	117	4·9	97	455
	3	1950	130	10·5	107	505
	4	2150	110	14·6	97	420
	5	2090	83	15·4	79	785
Total amounts excreted in "standard" sweats during a period of generous salt intake						
D.W.	1	2700	179	14·0	165	730
	2	2540	240	11·5	179	—
	3	3050	193	14·2	173	680
	4	3280	238	16·4	222	820
	5	3140	212	17·2	195	680
	6	3280	219	16·1	203	730
	7	—	—	—	—	—
	8	3740	254	18·6	230	810
R.A.M.	1	2100	120	6·2	110	370
	2	1750	147	8·5	124	560
	3	1810	127	8·6	117	480
	4	1980	143	10·5	130	560
	5	2090	153	10·5	149	530

1938]. Thus R.B.N. lost 2·44 g. of sodium on his first day's sweat and 1·65 on his last. J.T.B.'s daily loss of sodium declined from 4·95 to 2·6 g. Others fell off very much more. These results support those of Marchionini & Ottenstein [1931]—see also Glatzel [1937]. The potassium lost in the sweat remained constant in amount in some subjects and rose in others and the same may be said of the nitrogen. It follows that the sodium/potassium ratio underwent extensive alterations as the deficiency progressed. In Table VII may be found the results obtained on D.W. and R.A.M.

Daly & Dill [1937], Dill *et al.* [1933, 1936, 1937] and Talbott [1935] have all observed somewhat similar decreases in the concentration

of sodium chloride in successive sweats. They consider the phenomenon, however, to be part of the mechanism of adaptation which people, who are daily subjected to high temperatures, are said to undergo. They regard it as a provision of nature to safeguard the individual from excessive salt depletion, but they do not seem to have excluded the possibility that the falling concentration of sodium chloride might be a sign of salt deficiency rather than of adaptation. In order to find out what part adaptation might be playing in the results just described, D.W. and R.A.M. subjected themselves to a second series of sweats comparable in all respects with those which they had undergone during their deprivation experiment, except that throughout the control series they took daily an abundance of salt by mouth to replace the losses in the sweat. The results are given in Table VII. It is evident that as the days passed there was no falling off in the NaCl lost in the sweat and that in fact the reverse took place. It is also of interest to note that there was an increased excretion of potassium but not to the same extent as during salt deficiency. The present results therefore show that in these subjects repeated sweating did not lead to any reduction in the excretion of NaCl unless the repeated sweating was allowed to induce a state of salt deficiency. In that event the concentration of NaCl in the sweat invariably declined and it is concluded that salt deficiency and not "adaptation" was the cause. The arrangements in the present experiment were so different from those of Talbott and Dill *et al.* that the results can scarcely be said to contradict their conclusions. They do however show that the decreased secretion of NaCl observed in the present experiment was due to salt deficiency and not to adaptation.

Summary

Experimental sodium chloride deficiency in man produced:

(1) A reduction in the volume of the body fluids in which injected sucrose or inulin was dissolved (the extracellular fluids). In the present experiments reductions of 28–38 p.c. were observed.

(2) A fall in the concentration of sodium, a rise in the concentration of potassium and no consistent change in the concentration of chloride in resting saliva.

(3) A variable reduction or little change in the free and total HCl and in the total chloride of gastric juice. These changes bore no close relationship to the simultaneous chloride changes in the plasma. There was also a small fall in the sodium and rise in the potassium in gastric juice.

(4) A fall in both sodium and chloride of cerebrospinal fluid.

(5) A considerable but variable fall in the concentration of NaCl in sweat. It was shown that "adaptation" to repeated sweating did not contribute to this result.

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THE CARBOHYDRATE METABOLISM OF THE FŒTAL DOG UNDER THE INFLUENCE OF INSULIN

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When insulin is injected into the fœtus, and then only, a large amount of sugar passes from the mother to the fœtus [Schlossmann, 1931]. The object of this present paper is to show where this sugar goes. One possibility is that, when insulin is injected into the fœtus, the sugar coming from the mother may be stored in the liver of the fœtus, in which case the glycogen content of the fœtal liver might be expected to rise. Similarly glycogen might be stored in the fœtal muscle with a corresponding result. Alternatively the sugar may be converted into lactic acid. These possibilities have been investigated.

Methods

The experiments were carried out on pregnant dogs near term. The uterus was opened in a saline bath at 37° and the fœtuses were delivered into the saline as previously described [Schlossmann, 1932]. Using this method the fœtuses are kept under the surface of the saline throughout the experiment. Blood samples were taken from the umbilical vessels by lifting the cord just out of the saline. For anæsthesia the barbiturate "pernocton" (Riedel-de Haën, Berlin) was injected intravenously, as pernocton is known to affect the blood-sugar level less than most anæsthetics.

The blood sugar was estimated by the Hagedorn and Jensen method. The lactic acid was determined colorimetrically according to the method of Dische & Laszlo [1927]. This method needing only 1 ml. of blood was the only suitable one as the available volume of blood in the fœtal dog is small. Glycogen was estimated by Pflüger's method. In a number of experiments the total carbohydrate was determined instead of the glycogen: the value of these estimations has been discussed by Burghard & Paffrath [1927].

The glycogen content of the fœtal liver and muscle

The glycogen content of the fœtal liver and muscle is shown in Table I. In the first experiments no insulin was injected into the fœtuses. In the last experiments different amounts of insulin were injected intraperitoneally into some of the fœtuses 1–6 hr. before, the others being taken as controls. No significant difference was found between normal fœtuses and those into which insulin had been injected. The same result was obtained in two similar experiments on pregnant cats.

Table I. Glycogen or total carbohydrate content in g./100 g. in normal dog fœtuses and in fœtuses into which insulin was injected intraperitoneally

						Liver		Muscle	
No. of exp.	Day of pregnancy	No. of the fœtus	Units insulin injected	Hours after insulin injection	Hours after start of exp.	Glycogen	Total carbohydrate	Glycogen	Total carbohydrate
18	50	1	—	—	0·25	—	1·37	—	1·28
		2	—	—	0·25	—	1·31	—	—
		3	—	—	0·25	—	1·51	—	—
		4	—	—	0·25	—	1·53	—	—
35	57	1	—	—	0·25	5·56	—	—	—
		2	—	—	0·25	3·83	—	—	—
		3	—	—	0·25	4·48	—	—	—
38	60	1	—	—	0·25	4·18	—	—	—
		2	—	—	0·25	4·69	—	—	—
		3	—	—	0·25	2·64	—	—	—
		4	—	—	0·25	4·01	—	—	—
20	60	1	—	—	1·0	7·64	7·86	1·40	2·02
		2	20	5	6·0	8·22	—	1·40	1·67
		3	20	6	7·0	7·15	7·32	1·55	1·64
19	60	1	—	—	0·5	—	11·70	—	1·80
		2	60	1	2·0	—	8·22	—	1·74
		3	—	—	2·75	—	9·15	—	1·79
		4	60	2·5	3·5	—	9·75	—	2·26
		5	60	4	5·0	—	9·50	—	1·95
		6	—	—	5·5	—	9·61	—	1·41
		7	60	6	7·0	—	7·24	—	2·06
		8	—	—	7·5	—	6·96	—	—
		9	—	—	7·5	—	9·52	—	1·67

The well-known fact [Needham, 1931] that the glycogen content of the fœtal liver rises rapidly at the end of gestation and especially during the last few days before birth is also illustrated in my experiments. The values which I have found in fœtal dogs at the end of gestation are comparatively high.

Prolonged anæsthesia and the delivery into the saline bath did not markedly affect the glycogen content of the fœtal liver. On the other hand, the glycogen content of the maternal liver decreased gradually during the experiments, for instance in Exp. 20 from 1·75 to 0·06 g. p.c.

during 6 hr. The decrease in the maternal glycogen content is due to the operation and anæsthesia and not to the loss of sugar to the fœtus—small in relation to the maternal carbohydrate store—as the same decrease is found in experiments in which no insulin has been injected into the fœtuses.

Lactic-acid level of the fœtal blood before and after the injection of insulin

The lactic-acid level of the fœtal blood was determined in seven experiments on pregnant dogs before and after the intraperitoneal injection of high doses of insulin into some of the fœtuses. The experiments were in part identical with those mentioned in Table I.

The lactic-acid level was always substantially higher in the fœtal blood than in the maternal. This also holds for fœtuses from which blood samples were taken immediately after delivery into the saline bath. If no insulin were injected, the lactic-acid level of the fœtal blood remained constant for many hours. In these circumstances a rise in the lactic-acid level indicates that the normal placental exchange is disturbed in some way, generally by partial separation of the placenta.

After insulin was injected into the fœtus the lactic-acid level of the fœtal blood increased within a few hours. That this rise is not due to an impaired exchange through the placenta was proved by the contrast in the difference between the lactic-acid levels in the fœtal arterial and

Table II. Exp. 22. Dog 59 days' pregnant. Weight 32·0 kg. Pernocton anæsthesia. Nine fœtuses, of which five were each injected with 70 units of insulin (Hoechst) intraperitoneally. The values for blood sugar and lactic acid are given in mg. p.c. The umbilical vessels of fœtus 1 and 2 were punctured several times during the experiment. Figures in italics relate to fœtuses into which insulin had been injected.

Min. after insulin injection	No. of fœtus	Blood sugar		Lactic acid		Mother, jugular vein	
		Umb. artery	Umb. vein	Umb. artery	Umb. vein	Blood sugar	Lactic acid
Before	1	—	79	—	25·9	84	12·5
60	*2*	*72*	*75*	—	*37·9*	88	15·0
120	1	90	89	—	21·1	83	13·0
170	*2*	*49*	*54*	*28·8*	*25·9*	—	—
210	*3*	*53*	—	—	*40·8*	78	—
280	1	69	70	24·0	21·1	72	13·5
340	7	—	69	—	21·1	68	—
430	1	66	65	21·1	22·6	63	12·5
—	*2*	*35*	*62*	*57·6*	*43·7*	—	—
660	1	73	75	70·0	68·2	72	13·5

venous blood. In the normal fœtus the lactic-acid level was about 1 or 2 mg. p.c. higher in the blood from the umbilical artery than in that from

the umbilical vein. When the lactic-acid level rose during a prolonged experiment, the difference scarcely increased, as sufficient amounts of lactic acid could no longer pass across the placental barrier. But following the injection of insulin into the fœtus, the difference between the fœtal arterial and venous blood became much greater. This means that the arterial blood reaches the placenta loaded with lactic acid, a substantial portion of the lactic acid being delivered therefrom to the mother (see Table II).

The lactic-acid level in the maternal blood (jugular vein) varied only within small limits during the experiments. With one exception all maternal lactic-acid values lay between 10 and 20 mg. p.c.

Relations between sugar and lactic-acid levels of the fœtal blood after the injection of insulin

The relations between sugar and lactic-acid levels in the fœtal blood following the injection of high doses of insulin can clearly be seen from Figs. 1 and 2. In the experiment which is shown in Fig. 1, blood samples from the umbilical vein and from the heart were taken once only from each fœtus. The fœtuses were killed immediately afterwards in order to determine the total carbohydrate in liver and muscle (Table I, exp. 19). The blood lactic-acid level remained virtually unchanged for 7 hr. in the four fœtuses into which no insulin had been injected, and the corresponding values of the blood sugar were close to the line which is given by the maternal blood sugar. On the other hand, in the blood of the fœtuses to which insulin had been administered, the lactic-acid level rose and the blood sugar fell progressively during the experiment. The relation between decrease of sugar content and increase of the lactic-acid level in the fœtal blood after the injection of insulin into the fœtus can be seen still better from Fig. 2. In this experiment arterial and venous bloods of fœtus 2 were obtained at the beginning and end of a 100 min. period. The differences between the values for arterial and venous blood were much greater in the second samples, corresponding to the lower blood sugar and higher lactic-acid levels in the arterial blood.

The uptake of glucose by the fœtus and the passage of lactic acid to the mother can be calculated approximately on the basis of these experiments by the differences between sugar and lactic-acid levels in the blood of the umbilical vein and umbilical artery. The calculation in Table III shows that, under the influence of insulin, the fœtus obtained from the mother 143 mg. of glucose per hr. above the normal and, at the same time,

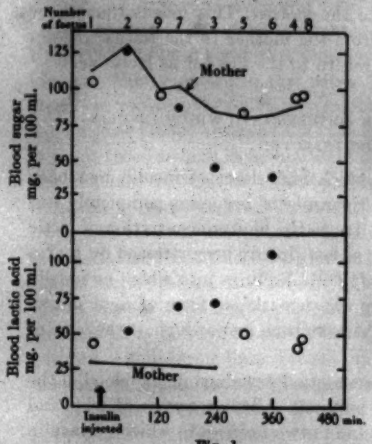
Number of foetus
1 2 9 7 3 5 6 4 8
Blood sugar mg. per 100 ml.
Mother
Blood lactic acid mg. per 100 ml.
Mother
Insulin injected
120 240 360 480 min.

Fig. 1.

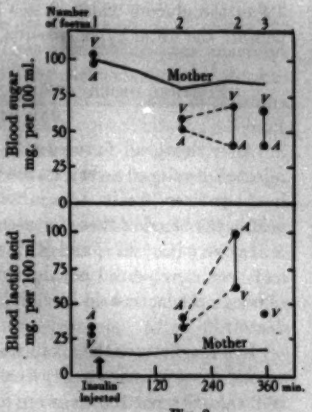

Fig. 2.

Fig. 1. Exp. 19. Dog 60 days' pregnant. Weight 27·2 kg. Pernocton anæsthesia. Nine fœtuses were delivered into the saline bath of which four (nos. 2, 3, 6, 7) were each injected intraperitoneally with 60 units of insulin (Hoechst). The dots represent values obtained from these fœtuses, whereas the circles represent values obtained from the five other fœtuses into which no insulin had been injected. The blood was taken by heart puncture, except from fœtuses 1, 2 and 3 (umbilical vein) and 7 (umbilical artery).

Fig. 2. Exp. 20. Dog 60 days' pregnant. Weight 17·6 kg. Pernocton anæsthesia. Three fœtuses were delivered into the saline bath. 20 units of insulin (Leo) were injected intraperitoneally into fœtuses 2 and 3. *V* = blood taken from umbilical vein. *A* = blood taken from umbilical artery, except the second sample of fœtus 2, where blood was obtained by heart puncture. The figures for the glycogen and total carbohydrate content of liver and muscle are shown in Table I.

TABLE III. Differences between amounts of sugar and lactic acid in blood of umbilical vein and umbilical artery

	Blood sugar (passing from mother to fœtus) mg. p.c.	Lactic acid (passing from fœtus to mother) mg. p.c.
Normal fœtus	3	1
Fœtus 3–5 hr. after injecting insulin	25	18
Increase	22	17
Corresponding to	143 mg. per hr.	111 mg. per hr.

[1] The assumption is made that the weight of the dog fœtus near term averages 300 g. and that about 650 ml. fœtal blood passes through each dog placenta per hr. [Schlossmann, 1931, 1932].

delivered 111 mg. of lactic acid to the mother. This means that about 3/4 of the glucose which passes from the mother to the fœtus to compensate the fœtal hypoglycæmia return to the mother as lactic acid.

Further observations on fœtuses into which insulin had been injected

After death all fœtuses into which high doses of insulin had been injected developed a very marked rigor mortis, becoming completely stiff in about 10 or 15 min. This is not due to the high concentration of lactic acid in the blood of these fœtuses, as has already been stressed by Reiss & Haurowitz [1929] and Reiss [1932]. Fœtuses into which no insulin had been injected did not develop this remarkable form of rigor mortis although the lactic-acid level might have been just as high at the time of death.

In a few fœtuses the alkali reserve was determined in the blood of the carotid artery by van Slyke's method. Alkali reserves of 26·9 and 28·8 vols. CO_2 per 100 c.c. were found in two fœtuses to which no insulin had been administered, immediately and 270 min. after delivery into the saline bath; whereas in two fœtuses, 180 and 270 min. after the injection of 60 units of insulin intraperitoneally, the alkali reserves had dropped to 11·0 and 21·0 respectively. The figures for the lactic-acid levels in these two fœtuses were 104 mg. p.c. for the former and 37·3 mg. p.c. for the latter.

Discussion

The experiments described above show that considerable amounts of lactic acid are formed from glucose under the influence of insulin, the glycogen content of the fœtal liver and muscle remaining virtually unaffected. The amount of lactic acid which passes after injection of insulin into the fœtus through the placenta to the mother almost corresponds to the increased amount of sugar which passes from the mother to the fœtus.

Indeed it is known that the lactic-acid level of the blood also rises in the adult after injection of insulin, the rise being proportional to the dose [Bürger *et al.* 1935]. Furthermore, the lactic-acid level and the excretion of lactic acid in the urine are increased after the infusion of glucose into dogs, and the increase is still higher if glucose and small amounts of insulin are infused together [Wierzuchowski & Laniewski, 1931]. But in the fœtal dog the formation of lactic acid is increased to a point which can only be explained by assuming a special reaction of the fœtal cells. Some corresponding observations by other authors will make this

clearer. In new-born mice and rats, which survived the inhalation of carbon monoxide for several hours, Reiss & Haurowitz [1929] and Reiss [1932] found nearly the same increase in the lactic-acid level of the blood as that reported in this paper. They pointed out that anaerobic glycolysis replaces the oxidations inhibited by carbon monoxide. Seckel & Sommer [1935] observed that the excretion of lactic acid in the urine is about twice as high in prematurely born as in normal infants, although the contrary should be expected considering the comparatively few muscular movements in the former. Moreover, it has been demonstrated by Schönfelder [1935] that the excised muscle of fœtal and new-born rabbits forms lactic acid from glucose in much greater amount than does that of adult rabbits. And recently Needham & Lehmann [1937] have shown that in the embryo generally a very active non-phosphorylating mechanism is present by which lactic acid can be formed from glucose without an intermediate formation of glycogen. After birth this mechanism is only found in certain tissues.

These observations show that glycolysis plays a more important part in the fœtal organism than in the adult. In the carbohydrate metabolism of the fœtus, glycolysis can be substituted for oxidative processes. Warburg [1926] has demonstrated that fœtal tissue glycolyses aerobically only under abnormal conditions, for instance when Ringer solution is substituted for serum. Bearing in mind the large lactic-acid formation from glucose after the injection of insulin, one might assume that insulin causes such abnormal conditions in the fœtus, the fœtal cells now converting glucose to lactic acid without further oxidation of the lactic acid. The amount of oxygen available in the fœtal blood is by no means sufficient to oxidize the lactic acid formed from glucose after injecting large doses of insulin. The complete oxidation of 143 mg. of glucose requires 107 c.c. O_2, whereas the total oxygen uptake of the fœtus—based on the figures shown in Table III—is about 30 c.c. per hr. Even if all the oxygen which passes through the placenta to the fœtus is used for the oxidation of glucose or lactic acid, only about 27 p.c. could be completely oxidized.

It has been stated in this paper that certain amounts of glucose pass from the mother to the fœtus, and of lactic acid from the fœtus to the mother. Strictly speaking, evidence has only been brought that the glucose and lactic-acid levels are different in the blood of the umbilical vein and umbilical artery. But it seems quite unlikely that any considerable amount of lactic acid could be converted into glucose in the placenta, therefore the above statement may safely be made.

Another fact which is shown by my experiments is the high tolerance of the fœtal dog to insulin. For rabbits this has been demonstrated previously by Snyder & Hoskins [1927], and according to Wertheimer [1930], new-born mice are completely resistant to toxic doses of insulin. Although I have injected up to 270 units of insulin into one fœtus (770 units per kg.), convulsions were never observed. The blood sugar fell more slowly than in adult dogs into which small doses of insulin were injected, and the decrease stopped when the blood-sugar level had dropped to about 40 mg. p.c. As long as the fœtuses are connected to the mother by the placenta and the umbilical cord, the glucose passing from the mother to the fœtus works, of course, like a therapeutic intravenous glucose infusion. It is clear that during this stage the blood sugar cannot drop below a certain level. But, also after tying off the umbilical cord and taking the fœtus out of the saline bath, some of the new-born survived for a period up to 2 hr. without showing convulsions, and without a further considerable drop in the blood-sugar level.

The independence of the fœtal glycogen stores has previously been shown by Wertheimer [1930], Huggett [1929] and Corey [1935]. The values in Table I demonstrate that in the fœtal dog the glycogen stores are scarcely affected by prolonged experiments under pernocton anæsthesia or by high doses of insulin, while the glycogen content of the maternal liver becomes very low.

Summary

In the fœtal dog the glycogen or total carbohydrate content of liver and muscle is not influenced by the intraperitoneal injection of high doses of insulin. The fœtal dog is highly resistant to insulin.

The lactic-acid level of the fœtal blood rises a few hours after the injection of insulin. The difference between the lactic-acid levels in the blood of the umbilical artery and umbilical vein is increased many times above the normal. About 3/4 of the glucose which passes from the mother to the fœtus in order to compensate the fœtal hypoglycæmia is returned to the mother in the form of lactic acid.

Under the influence of insulin the fœtal carbohydrate metabolism is characterized by a high rate of aerobic glycolysis.

It is a pleasure to thank Sir Joseph Barcroft for advice and for hospitality in his laboratory.

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SPECTROPHOTOMETER INVESTIGATION INTO THE DIFFERENCES BETWEEN FŒTAL AND MATERNAL HÆMOGLOBIN IN MAN

By J. JONGBLOED

From the Physiological Laboratory, Utrecht

(*Received* 14 *September* 1936)

It has been shown that in different animals fœtal hæmoglobin has a higher affinity for oxygen than has that of the mother [Barcroft, 1934]. When the dissociation curves are compared the fœtal curve is found to lie to the left of that of the mother. The difference might be explained either by assuming that the maternal differs from the fœtal hæmoglobin or that in the same blood there is more than one sort of hæmoglobin, the proportions of the two forms being different in the fœtal and maternal bloods. Von Krueger & Bischoff [1925] and Brinkman [1934] have shown that the resistance towards alkaline denaturation differs in maternal and fœtal hæmoglobin.

The few dissociation curves which have been recorded on men at high altitudes also seem to be shifted to the left [Barcroft, 1934], and it was suggested that oxygen want tends to displace the adult dissociation curve to its pre-natal position. As this would be a very interesting fact if true, I looked for a simple physical method of demonstrating the difference between these kinds of hæmoglobin for later use in altitude experiments.

For this purpose I attempted to demonstrate the difference between maternal and fœtal hæmoglobin by direct examination of their respective spectra in the ultra-violet. Five experiments were performed and, so far as can be judged by photometric examination of the records, there was no material difference between the maternal and fœtal hæmoglobin. Although the result was negative I feel that it may be of interest to other workers on this subject. An example of the method used is given in the following protocol.

Exp. No. 5. 9 *December* 1934. Human blood obtained from the mother by puncture of the cubital vein some hours before parturition. Fœtal blood obtained from the umbilical cord during birth. A weak solution of hæmoglobin was made up, first washing the corpuscles repeatedly with saline and then hæmolysing them with distilled water. The solutions were saturated with oxygen from the air and distilled water was added until both were of exactly the same depth of colour. A Hilger all-metal quartz spectrograph with hydrogen source was used. The time of exposure was 1 min. The thickness of the absorbing layer of hæmoglobin was varied by a Baly tube.

A series of spectra from the maternal hæmoglobin solution was first taken, followed on the same plate by corresponding spectra from the fœtal solution. The absorptions seem to be the same in both cases (Fig. 1), but for finer analysis I measured the records with a Kipp photometer by two methods. The records obtained by the first method are shown in Fig. 2, where the upper curve refers to the maternal hæmoglobin and the lower curve to the fœtal hæmoglobin. It will be seen that the two curves are almost identical. In the second method the blackening of the plate at a wave-length of 3850 A. was measured by the optical density. The density of each strip for both kinds of hæmoglobin is indicated on the graph. It will be seen that there is no difference between the densities of the corresponding strips (1–5, Fig. 3).

In one experiment I used the bloods of twins, both of which were found to be identical with the maternal blood by the methods used in this paper.

Discussion

Haurowitz [1935] has shown that a solution of hæmoglobin from a newborn child has less affinity for oxygen than a similar solution obtained from the mother. A suspension of fœtal corpuscles, on the other hand, has a greater affinity for oxygen than one of the maternal corpuscles. This reversal of the positions of the dissociation curves does not exist in the experiments which Barcroft has made on different animals. The special property of human hæmoglobins which has been shown to exist by Haurowitz does not affect the point of my experiments, since I was attempting to find out whether the *difference* in affinity for oxygen gives in addition a difference in the short-wave spectrogram.

Purer solutions of hæmoglobin than those used would have been preferable. It is also possible that hæmoglobin from a younger fœtus would have been different from the maternal hæmoglobin. Nevertheless one would have expected to find some slight difference in these experiments if the difference with a younger fœtus was considerable. I am

Fig. 1.

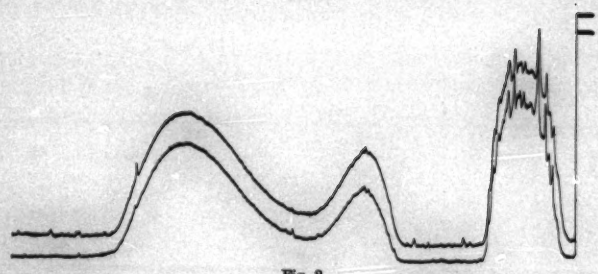

Fig. 2.

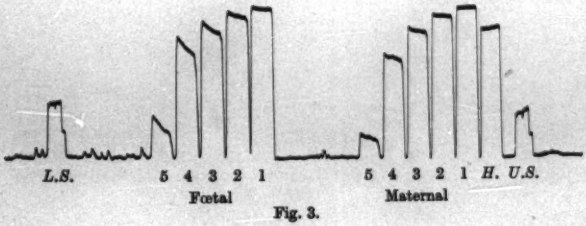

Fig. 3.

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inclined to conclude that the difference in the affinity for oxygen of maternal and fœtal hæmoglobin in man is not correlated with a demonstrable difference in absorption in the ultra-violet.

Summary

Absorption curves in the ultra-violet were obtained from fœtal and maternal hæmoglobin in man. Although the oxygen affinities of the two kinds of hæmoglobin are different from one another, there is no demonstrable difference in their absorption curves.

I am greatly indebted to Sir Joseph Barcroft who was kind enough to read the manuscript and to give me valuable advice. I am also indebted to Prof. A. K. M. Noyons, the Director of the laboratory in which the work was carried out.

The Editorial Board regrets that owing to an oversight there has been delay in the publication of the above paper.

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EXPLANATION OF PLATE I

Fig. 1. Uppermost: hydrogen-spectrum. Next, eight spectra of maternal hæmoglobin, thickness of the layer respectively 5, 10, 15, 20, 30, 40, 50 and 60 × 0·85 mm. Lowermost: eight spectra of fœtal hæmoglobin with same thickness of layer.

Fig. 2. Photometrical analysis of the sixth spectrum of Fig. 1 from maternal (upper curve) and fœtal (lower curve) hæmoglobin.

Fig. 3. Transverse photometrical analysis of Fig. 1 at a wave-length of 3850 A. (i.e. midway between 38 and 39 on the scale). *L.S.* lower scale (to be neglected). Fœtal 5–1: first five spectra fœtal hæmoglobin. Maternal 5–1: first five spectra maternal hæmoglobin. *H.* hydrogen spectrum; *U.S.* upper scale (to be neglected).

Mean height of galvanometer deflexion on original graph:

Spectrum	Fœtal mm.	Maternal mm.
5	12	9
4	45	42·5
3	54	54
2	60	60
1	63	63·5

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ISOTONIC EXTENSION AND SHORTENING OF THE ANTERIOR RETRACTOR OF THE BYSSUS OF *MYTILUS EDULIS*

By INDERJIT SINGH

From the Physiological Laboratory, Cambridge

(*Received* 29 *November* 1937)

Isotonic extension and shortening of unstriated muscle have been described by Jordan [1929] and Winton [1930]. In this paper the effect of certain ions and drugs on isotonic extension and shortening of *Mytilus* plain muscle is described.

The muscle was stretched or released from a stretch by a method described by Winton [1930], with the modification that in some critical experiments the muscle was connected to the lever by steel piano wire and as far as possible thread was eliminated between the attachments. Arrangement for electrical stimulation of muscle was as described previously [Singh, 1938].

Results

Skeletal muscle has been represented mechanically by two springs, one undamped and the other damped in a viscous medium [Levin & Wyman, 1927]. The stretch and release curves of skeletal muscle are exponential; unstriated muscle on the other hand presents a viscous phase during which it stretches with a uniform velocity [Winton, 1930].

One of the differences between an isolated striated muscle and an isolated unstriated muscle is that the latter is usually found in a state of contracture or tone. So it was conceived that the difference between the extension curves of striated and unstriated muscle might be due to the presence of tone in the latter; accordingly, extension curves were recorded of frog sartorius in which contracture had been produced by caffeine (Fig. 1), sodium thiocyanate or barium. Caffeine was found to be the most effective substance. It will be seen that the extension of frog sartorius in caffeine contracture presents a viscous phase with a terminal visco-elastic phase, as in extension of dog retractor penis.

That the viscous phase represents relaxation of tone is also shown by the following experiments. Muscles were made to contract with potassium (0·1 *M* KCl) using an isotonic lever, lifting a weight of 9 g. When they

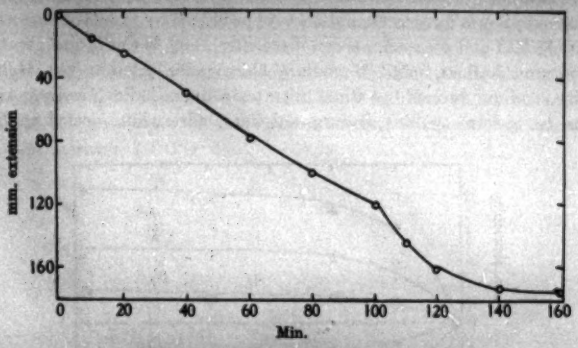

Fig. 1. Extension of frog sartorius in contracture induced by 1 in 200 caffeine. Stretching load 18 g.; magnification by lever 7 times.

Fig. 2. The muscle was immersed in 0·01 *M* KCl (in *Mytilus* saline) at *K*. It contracted (loaded with 9 g.) and then began to relax. As it was relaxing the smoked paper was set in motion, and the load was released at *A*; the muscle was again stretched at *B*.

started relaxing after contraction, the weight was released. After a short time the weight was again dropped on to the lever, and the viscous phase of the extension curve was found in line with the curve of relaxation (Fig. 2).

All substances that cause the muscle to contract produce curves which show decreased rate of extension and increased rate of shortening; all substances that diminish tone produce the opposite effect, that is, increased rate of extension and decreased rate of shortening (Fig. 3). Curves similar in form to 2*a* and 2*b* were produced by excess of potassium (0·03 *M* KCl and greater), excess of calcium (0·03 *M* $CaCl_2$ and greater), strontium, barium, 0·564 *M* sodium chloride or calcium-free *Mytilus* saline, sodium bromide, sodium nitrate, sodium iodide, sodium thiocyanate, sodium cyanide, sodium sulphate, adrenaline, acetyl choline,

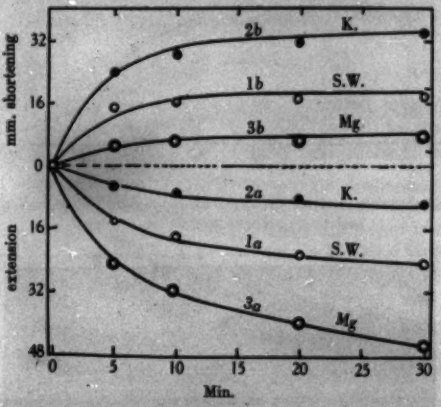

Fig. 3. Effect of potassium and magnesium on extension and shortening. Curves 1*a*, 1*b*, in sea-water; curves 2*a*, 2*b* in potassium-rich sea-water (20 c.c. of 0·564 *M* KCl + 80 c.c. of sea-water); curves 3*a*, 3*b* in magnesium-rich sea-water (20 c.c. of 0·376 $MgCl_2$ + 80 c.c. of sea-water). Immersion for 30 min.

veratrine, caffeine. Curves similar in form to 3*a* and 3*b* were produced by calcium (0·01–0·02 *M* $CaCl_2$) and magnesium in all concentrations, which diminish tone. The first set of curves cannot be due to an active contraction, i.e. a change in the unloaded length of the muscle, since such an effect by potassium as described above may be produced 4–6, sometimes even 12 hours, after immersion of the muscle in a potassium-rich solution. I have never known a contraction produced by potassium to last so long. The longest time I have known for potassium to produce a rising tension (not relaxation) is 30 min., and in the presence of potassium the tension subsides in 15–75 min. Moreover the minimum

concentration of potassium which produces contraction is about 0·05 *M* KCl, while the above effect may be produced by excess potassium and calcium in concentrations as low as 0·03 *M* KCl, or $CaCl_2$, a concentration that has never produced a contraction. It would appear, therefore that asymmetric changes are produced in the constants of the visco-elastic system, so that their magnitudes are different according to whether the muscle is shortening or lengthening. Such an effect recalls the "catch" mechanism of Grützner [1904] and v. Uexküll [1912]. If such a substance as potassium which decreases the rate of extension is removed, the rate increases (cf. Fig. 6).

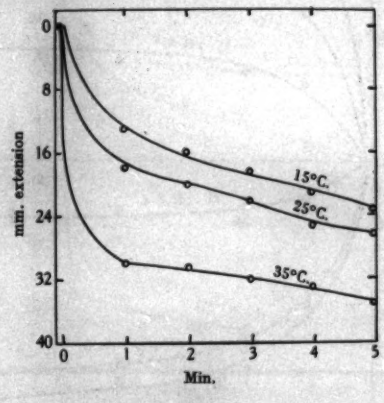

Fig. 4. Effect of temperature on extension.

If the muscle is naturally insensitive, or artificially rendered insensitive, to potassium by exclusion of sodium chloride (replacement with glucose) or calcium, then smaller concentrations of potassium (0·01 *M* KCl and upwards) increase the rate of extension. Adrenaline also increases the rate of extension if the muscle is naturally insensitive or artificially rendered insensitive by the above means. If the muscle is immersed in sea-water or *Mytilus* saline, then subliminal concentrations of drugs such as adrenaline (1 in 10^7–10^8), eserine (1 in 2×10^5), veratrine hydrochloride (1 in 1500), and caffeine (1 in 100) increase the rate of extension. Increase of temperature (experimental range 15–35° C.) increases the rate of extension (Fig. 4).

Certain substances, however, increase or decrease both the rate of extension and the rate of shortening, so that these substances may be said to produce symmetrical changes in the visco-elastic system of the muscle. Decrease in *p*H to 5–4·4 produces a great decrease in the rate of change of length which is reversible (Fig. 5). Excess of sodium chloride (0·1–0·5 *M*) added to *Mytilus* saline decreases, while reduction

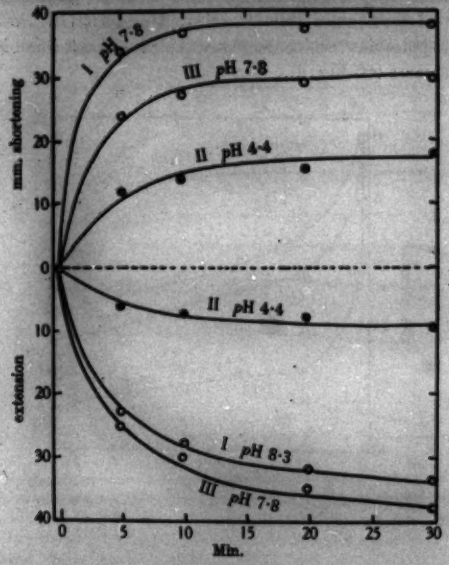

Fig. 5. Effect of *p*H 4·4 (*Mytilus* saline) on extension and shortening. Time of immersion in each solution 1 hour.

in the sodium chloride content (hypotonic solution of osmotic pressure 60–70 p.c. of normal) increases the rate of change of length. Muscles stored in the refrigerator for 2–3 weeks, those from cooled animals, and those immersed in *Mytilus* saline (at *p*H 7·8, *M*/300 borate buffer) for some hours change in length slowly. Under these conditions the muscle also contains excess of sodium and chloride [Singh, 1938]. The sodium content of frog muscle increases as a result of activity [for discussion, see Fenn, 1936]; Levin & Wyman [1927] found that the viscosity of frog muscle is increased during fatigue.

The following substances produce a great increase in the rate of change of length: excess potassium or ammonium (0·4–0·5 *M*), adrenaline (in muscles which do not contract), veratrine hydrochloride (1 in 1000, *p*H 7), hypotonic solutions (0·6 p.c.) of sodium and potassium chlorides. Isotonic solutions of glucose and hypotonic solutions of calcium and magnesium chlorides (0·6 p.c.) produce a fall in the rate of change of length, while the action of potassium and ammonium is counteracted by a decrease in *p*H to 5–4·4. Asymmetrical curves are produced by small

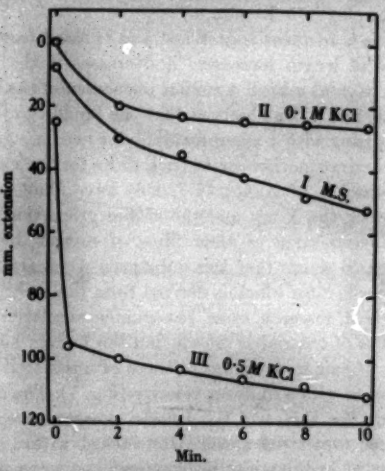

Fig. 6. Effect of potassium on extension. Immersion for 20 min. M.S. = *Mytilus* saline.

concentrations of potassium, as mentioned above, and by adrenaline if the muscle contracts. The rate of change of length of freshly dissected muscles may be very small; it increases greatly if they are left undisturbed in sea-water for 1–2 hours, and diminishes again after 12–16 hours; the response to A.C. is then very sluggish.

Smaller concentrations of potassium (0·1 *M*) at first decrease and then after some hours (10–12) increase the rate of extension; the greater the concentration of potassium, the sooner the rate increases; with 0·56 *M* it increases enormously within a few minutes (Fig. 6). Calcium (0·01–0·02 *M*) partly counteracts this action of potassium. Ammonium (0·05 *M*

and upwards at *p*H 7) antagonizes the effect of potassium, sodium and barium, if they decrease the rate of extension; excess of potassium antagonizes the effect of all substances mentioned above except that of *p*H. Adrenaline (1 in 10^6–1 in 5×10^4) antagonizes the effect of acetylcholine (1 in 10^6–1 in 5×10^4), caffeine (1 in 1000), veratrine hydrochloride (1 in 1000). Veratrine antagonizes the effect of acetylcholine, caffeine. Nicotine (1 p.c. at *p*H 7·8) antagonizes the effect of acetylcholine. Urea (5–10 p.c.) antagonizes the effect of glucose. When these various substances are added together, their action is synergistic; antagonism only comes into play in 5–10 min.

If the muscle is frequently stretched and released from a stretch, its rate of change of length increases; it decreases if the muscle is left undisturbed in *Mytilus* saline. A similar phenomenon has been described by Chambers [1921]. He observed that the liquidity of protoplasm is increased by stirring with a micro-manipulator needle. After some time the resistance against motion comes back to its former value.

Recent researches of Astbury [1933] have shown a remarkable similarity between the X-ray and the elastic properties of muscle and hair. X-ray investigation of their films of myosin by Astbury & Dickinson [1935] shows that this substance when stretched behaves very much like α-keratin which is derived from the hair.

In the present research some remarkable similarity between the elastic properties of unstriated muscle and the human hair were found. Stretch curves of hair were similar to those of unstriated muscle with a phase of extension with a uniform velocity (Fig. 7). The close similarity will be noticed. The hair, like the muscle, presents two phases during extension. When unstriated muscle is stretched with a series of loads which increase in arithmetical progression, the extension sometimes increases more rapidly up to a certain load. Thus isotonic stretch curves of a dog retractor penis [Winton, 1930] show an extension of 2 mm. in 50 min. with 2½ g. load; 3·5 mm. with 5 g. load and 24 mm. with 12½ g. load. Extension of a human hair in 30 sec. was 2 mm. with a load of 20 g., 5·5 mm. with 30 g. and 13 mm. with 40 g. Often the recovery of unstriated muscle takes a much shorter time than the extension to the same length. Thus a muscle which may take an hour to extend to a certain length may recover to its original unstretched length within 5 min. Similarly in the case of hair, where it may take about 30 min. to extend to a certain length, recovery of its original length may occur in a minute. This suggests that movement in one direction is facilitated and retarded in the other, something analogous to the behaviour of muscle in extending

and shortening in potassium-rich solutions. The rate of extension of hair as well as of muscle increases with temperature. Hair also shows an increase in the rate of extension if frequently stretched and released.

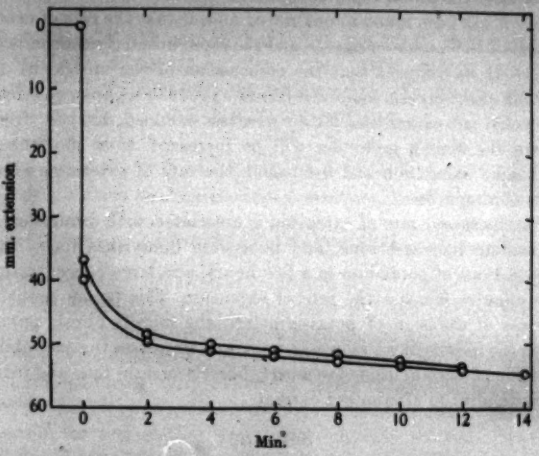

Fig. 7. Extension of *Mytilus* plain muscle (upper curve) and human hair (lower curve (load 18 g.)). Extension of both begins at the same point.

Discussion

Isotonic extension curves of *Mytilus* unstriated muscle are influenced by two factors: (*a*) visco-elasticity, (*b*) relaxation of contracture, either spontaneous (probably produced by sodium [Singh, 1938]) or produced by other substances such as potassium. The sudden and gradual extension of muscle when it is isotonically loaded may be explained on the same basis as the extension of gelatine, etc. [Busse, 1932]. The elastic properties of colloid systems require, and are produced by, (*a*) long fibrous molecules, (*b*) weak or uniform cohesive forces around the fibres, (*c*) an interlocking of fibres, (*d*) a means of storing up energy when the fibres are distorted.

The long fibrous molecules in the case of muscle may be said to be provided by myosin. The rapid initial elongation of muscle may be explained as due to the movement of kinky myosin molecules past one another owing to relatively weak cohesive forces. They move so far that some parts become jammed or tangled in intermediate equilibrium

positions. The energy of thermal vibrations would then cause the jammed parts of the molecule to escape slowly from these temporary equilibrium positions and become at least partly untangled, thus producing the slow stretch after the initial rapid elongation.

Now it has been found in unstriated muscle that the rate of extension decreases if the muscle contracts, and the rapid initial elongation becomes smaller. If we suppose that the contraction of the muscle be due to wrinkling of the myosin molecules [Astbury, 1933–4], then on stretching these would jam earlier than if they were less wrinkled, and the "friction" between the sliding molecules will be increased, thus shortening the initial rapid elongation and decreasing the rate of extension when the muscle contracts.

If an increased rate of extension is associated with diminished tone, then sodium increases tone, and potassium diminishes tone. Smaller concentrations of potassium in a few hours, and large concentrations in a few minutes increase the rate of extension. This points probably to a process of diffusion of potassium into the muscle fibres; potassium outside the muscle fibres increases tone, and decreases the excitability to A.C., while potassium inside the muscle fibres decreases tone and increases the excitability to alternating current.

Summary

1. Isotonic extension curves of *Mytilus* plain muscle resemble those of frog sartorius stretched while in contracture.

2. Substances that make the muscle contract decrease the rate of extension and increase the rate of shortening.

3. Certain substances increase or decrease the rate of extension as well as that of shortening.

4. Stretch curves of unstriated muscle resemble those of human hair.

I wish to thank Dr F. R. Winton and Dr S. L. Cowan for their help in this work.

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THE EFFECT OF ADAPTATION TO ELECTRICAL AND CHEMICAL STIMULATION ON THE EXCITABILITY OF THE ANTERIOR RETRACTOR OF THE BYSSUS OF *MYTILUS EDULIS*

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(*Received* 29 *November* 1937)

If the nerve free end of a frog sartorius muscle is stimulated by alternating current of fairly high frequency, the muscle contracts and quickly relaxes although stimulation is continued: in experiments which are made at one frequency, relaxation occurs earlier with some muscles than with others; the factor favouring early relaxation seems to be a high calcium ion concentration [Coppée, 1934]. If frog muscle is caused to contract by immersion in tetramethyl ammonium chloride solution [Marshall, 1914], the form of response depends on the calcium ion concentration present. If it is large the response is small and subsides rapidly, whereas if the calcium ion concentration is small the response is greater and maintained for a longer time. Wachholder & Matthias [1933] described the effect of calcium ions on the form of contracture of the frog sartorius when immersed in acetylcholine solution. *Mytilus* unstriated muscle also relaxes during continued electrical stimulation (A.C.) or chemical stimulation. The muscle in some way becomes adapted to the stimulus and in this paper the effect of certain substances on such adaptation is described.

The method used for electrical stimulation was the same as described previously [Singh, 1938]. The tension produced in the control solution (*Mytilus* saline) subsided to zero within 1–2 min.; the muscle in the experimental solution continued to contract, so that a rising tension was obtained. Changes in viscosity thus do not invalidate the results, which are qualitative. For chemical stimulation the muscle was immersed in the stimulating solution till it relaxed completely. A steady state was at first produced by stimulating the muscle three times at intervals of

1–1½ hours, depending upon the time of immersion of the muscle in the previous solution; the action of the experimental solution was then tried. An isometric lever was usually employed, but in some experiments an isotonic lever was used.

Results

Only those results are described which showed a marked difference between the action of the control and the experimental solutions.

In winter, muscles are usually more sensitive to potassium and less sensitive to A.C. than in summer. 0·1 *M* KCl in winter usually produces a 30–40 g. tension, and the muscle can also be stimulated by 0·05 *M* KCl (producing about 20 g. tension); A.C. (10 V.–10 sec.) produces a 20–30 g. tension. In summer the muscle is usually inexcitable to 0·05 *M* KCl and

Fig. 1. Effect of continuous stimulation with A.C.

0·1 *M* KCl produces 5–10 g. tension; A.C. (10 V.–10 sec.) produces 40–50 g. tension. In these muscles a continuous tension may be produced by 8–16 V. A.C. for 5 min. In June 1936, muscles with similar characteristics as described above were found, but there were a few batches of animals, the muscles from which were exceptionally insensitive to potassium. Some of these muscles required as much as 0·2–0·25 *M* KCl to stimulate them. Their sensitivity to A.C. was also low, 10 V.–10 sec. producing 5–15 g. tension. About 30–40 V. was required to produce a continuous tension (Fig. 1). The concentration of potassium could be raised to 0·2 *M* KCl in 20–30 min. without causing stimulation, though ordinarily it is difficult to raise the concentration to the same value in 1 hour without causing stimulation. When stimulated with A.C. (8 V.–10 sec.) fatigue was rapid, the muscle becoming inexcitable after four or five contractions; when stimulated with potassium, fatigue was similarly rapid and the muscle became inexcitable to 0·2 *M* KCl after three or four contractions. The muscle remained inexcitable to 0·1 *M* KCl before, during and after stimulation with A.C. (10 V.–10 min.) and during fatigue

following frequent stimulation with A.C. The response to D.C. resembled the response to A.C.

Response to electrical stimulation. In *Mytilus* saline (containing 0·01 *M* $CaCl_2$), if the muscle is stimulated with A.C. (10 V.–5 min.), the tension, which may reach about 20–30 g., subsides to zero in about a minute. In the absence of calcium, the tension during the first minute may be small, but it may continue to rise during the period of the passage of the current. If, in *Mytilus* saline, the tension subsides to zero with 8 V., and afterwards the voltage is increased to 16, and 24 V. at intervals

Fig. 2*a*. Continuous stimulation with A.C. steady state in *Mytilus* saline.

Fig. 2*b*. The same in Ca-free *Mytilus* saline.

of 3 min. respectively, the tension may again subside to zero. In the absence of calcium the tension may not be maintained with 8, or 16 V., but a rising tension is obtained with 24 V. (six experiments, Fig. 2, *a*, *b*). The tension produced by 8 V., however, is larger in the presence of calcium than in the absence of calcium. The effects of magnesium (0·05 *M* $MgCl_2$) and potassium (0·01–0·02 *M*) are similar to that of calcium.

Barium, sodium thiocyanate, sodium cyanide (Fig. 3), excess of sodium chloride (if 0·3 *M* is added to *Mytilus* saline), increase of temperature (experimental range 0–40° C.) (Fig. 4, *a*, *b*), have effects opposite to that of calcium. Barium and cyanide are the most powerful substances

in this respect. Freshly dissected muscles from cooled animals or from animals long stored in the refrigerator [Singh, 1938] may also not relax with continuous stimulation with A.C.; if these muscles are treated with a sodium chloride deficient solution (replacement of 30–40 p.c. of sodium chloride of *Mytilus* saline with osmotically equivalent amount of glucose

Fig. 3. Continuous stimulation with A.C. 9 V.–5 min.: (1) in *Mytilus* saline, (2) in 1 p.c. NaCN in *Mytilus* saline.

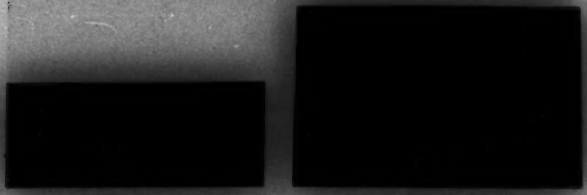

Fig. 4a. Fig. 4b.

Fig. 4a, b. Continuous stimulation with A.C. 9 V. Effect of temperature.

or a hypotonic solution), relaxation is hastened. The sodium content of such muscles is high [Singh, 1938]. In such muscles as mentioned above, the tension declined to zero with continuous stimulation with A.C. if the sodium chloride content of *Mytilus* saline was reduced to 60–70 p.c.; but with a smaller reduction of sodium content, the muscle contracted intermittently. Similarly, if in the presence of calcium the tension declined to zero with continuous stimulation with A.C., intermittent contractions were obtained in the absence of calcium if a rising tension was not

obtained. If with 0·02 *M* $CaCl_2$, the tension declined to zero, and in the absence of calcium the tension rose, then with 0·01 *M* $CaCl_2$ intermittent contractions were obtained.

Response to chemical stimulation. The muscle relaxes very slowly in solutions of 0·56 *M* NaCl, NaBr, $NaNO_3$ or *Mytilus* saline containing barium (0·04 *M* $BaCl_2$ and upwards, Fig. 5) or sodium cyanide (0·1 *M* NaCN and upwards). These substances prevent relaxation of the muscle when continuously stimulated with A.C. 0·01 *M* KCl and $CaCl_2$ hasten

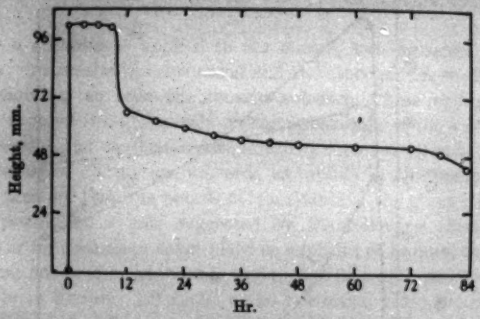

Fig. 5. Contraction produced by 0·07 *M* $BaCl_2$; the muscle lifted a weight of 10 g. Muscle was irritable for about 8 hours. Magnification by the lever 7 times.

relaxation of the contraction produced by sodium chloride, bromide, or nitrate; 0·01 *M* KCl and $CaCl_2$ also hasten relaxation when the muscle is continuously stimulated with A.C.

In small concentrations of potassium (0·05–0·1 *M* KCl), the muscle relaxes in 15–75 min. The higher the concentration of potassium, the sooner does the muscle relax. Calcium (0·01–0·02 *M* $CaCl_2$) opposes the action of potassium, so that in the absence of calcium relaxation is quicker. Contraction produced by isotonic solution of potassium chloride or sodium citrate subsides in a few minutes. Excess of sodium chloride (0·05–0·3 *M*) added to *Mytilus* saline greatly prolongs the relaxation of the contraction in potassium (1–3 hours). Replacement of the sodium chloride of the *Mytilus* saline with sodium bromide or sodium nitrate has a similar effect. Decrease in *p*H (Fig. 6) and increase in temperature (experimental range 14–40° C.) hasten relaxation. At about *p*H 6·5 potassium may only cause relaxation; at a temperature of 30–35° C.,

the potassium contraction, giving 10–15 g. tension, subsides within 30–40 sec. The longer the time taken to raise the concentration of potassium to a certain value, the smaller the tension produced, so that stimulation can be avoided by gradual increase in the concentration of potassium.

The contraction produced by acetylcholine at *p*H 7–6·5 in eserine (1 in 200,000) subsides in 1–2 hours; while that produced by adrenaline (1 in 50,000) or veratrine hydrochloride (1 in 1000) subside in 5–15 min.

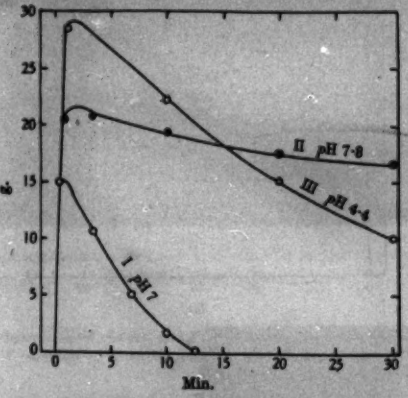

Fig. 6. Effect of *p*H on tension produced by 0·1 *M* KCl.

If the muscle is stimulated simultaneously with two substances in one of which it relaxes slowly and the other rapidly, the latter substance antagonizes the effect of the former substance. Thus the muscle relaxes more rapidly in adrenaline (1 in 10^8) and acetylcholine (1 in 10^8) than in acetylcholine alone; in barium (0·04 M $BaCl_2$) and potassium (0·4 *M*) than in barium alone; in sodium chloride and potassium chloride than in sodium chloride alone.

If the substance in which the muscle relaxes less rapidly has already produced a contraction, then the introduction of substances in which the muscle relaxes more rapidly, hasten relaxation of the contraction produced by the former substance. This resembles the effect of A.C. on D.C. contraction [Winton, 1937]. Thus potassium (0·4 *M*) or ammonium (0·4 *M*) neutralizes contractures produced by sodium, barium, calcium

and strontium chlorides; adrenaline neutralizes contractures produced by acetylcholine, veratrine, caffeine and sodium chloride; veratrine neutralizes contractures produced by caffeine and acetylcholine.

Discussion

Relaxation with continuous stimulation with A.C. or potassium is probably due to adaptation of the muscle to the stimulus, as shown by the fact that a sudden increase in the stimulus causes a further contraction; the process can be repeated with A.C. till a continuous tension is produced.

When a stimulus is applied to the muscle, two processes occur in the latter; one results in contraction and the other produces adaptation to the stimulus, so that the tension subsides. These two processes probably proceed simultaneously on the application of the stimulus, so that the resulting contraction is determined by these two opposing factors. If the process of adaptation proceeds as rapidly as the process which produces tension, then the muscle is inexcitable to the given stimulus.

This possibility is also suggested by the following observations: (1) Little or no relaxation takes place in solutions of barium, that is the muscle does not become adapted to barium and no muscle has been found inexcitable to barium. (2) Little or no relaxation takes place in high concentrations of sodium cyanide (0·1–0·2 *M*; though the muscle may relax in potassium cyanide owing to the potassium) and no muscle has been found inexcitable to such a high concentration of sodium cyanide. (3) The muscle relaxes slowly in acetylcholine, and rapidly in adrenaline, veratrine and caffeine; the muscle is most sensitive to acetylcholine. In six eserinized summer muscles, 1 in 10^8 acetylcholine produced 20–25 g. tension; the same concentration of adrenaline, caffeine and veratrine were ineffective. (4) In summer muscles sensitivity to potassium rises as the temperature is increased from 6° to 15° C. (40–50 g. tension); as the temperature is increased to 33° C., adaptation to potassium becomes very rapid, so that the potassium contraction resembles an A.C. twitch, but the sensitivity (tension) declines (10–15 g. tension). (5) With lowering of *p*H from 7·8 to 6·5, the muscle becomes adapted more rapidly to potassium and the above mentioned drugs; the sensitivity of the muscle to these substances also diminishes.

Though potassium is a more powerful stimulant than sodium, owing to rapidity of adaptation, the tension produced by 0·564 *M* KCl subsides in 5–10 min., while that produced by 0·564 *M* NaCl persists, so that ultimately a high tension (50–60 g.) may be produced by the latter.

Similarly the muscle gradually contracts to its maximum extent in barium or cyanide, and may die contracted.

Most substances have opposite effect on the excitability to A.C. and to potassium, but high concentrations of cyanide increase the excitability to A.C., D.C., potassium, adrenaline and stretch; while barium has no depressant action on the excitability to A.C. This increased excitability to A.C. in barium or cyanide is probably due to diminished adaptation.

Though a rising tension is produced in the absence of calcium, in sodium thiocyanate and sodium cyanide, the initial tension is smaller with these substances than in *Mytilus* saline. The initial tension may be said to be dependent upon the initial threshold, and the subsequent tension on the rate of rise of threshold. Thus calcium may be said to lower the initial threshold for A.C., but increases the rate of rise of threshold; sodium thiocyanate may be said to raise the initial threshold, but diminishes the rate of rise of threshold. The potassium contraction also shows a similar effect (Fig. 6); with *p*H 4·4 the tension produced is greater, but adaptation more rapid than with *p*H 7·8.

Summary

1. Sodium chloride, sodium cyanide, barium chloride, and increase of temperature, decrease adaptation to the stimulus of alternating current.

2. Calcium (0·01–0·02 *M* $CaCl_2$), magnesium (0·04 *M*), and potassium (0·01–0·02 *M*) increase adaptation to the stimulus of alternating current.

3. Adaptation to sodium chloride, sodium cyanide, barium chloride, is slow.

4. Adaptation to potassium is decreased by sodium chloride, sodium bromide, sodium nitrate, and calcium ions (0·01–0·02 *M*), and increased by rise of temperature (15–35° C.) and decrease of *p*H.

I wish to thank Dr F. R. Winton and Dr S. L. Cowan for help in this work.

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SOME CONDITIONS OF FŒTAL RESPIRATION IN THE COW

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A. THE OXYGEN CAPACITY

THE oxygen capacity of the maternal and of the fœtal blood is one of the conditions important for fœtal respiration, since the volume of gas available for the fœtus at a given oxygen pressure on either side of the placenta depends on it.

There is not much literature on this subject. In man one experiment, relating to a fœtus of 7 months, was made by Haselhorst & Stromberger [1930] who also give figures for four normal children immediately after birth. The results are collected here.

Age of fœtus	Weight in g.	O_2 capacity of fœtal blood in vol. p.c.	O_2 capacity of maternal blood in vol. p.c.
7 months	1700	18·14	17·26
At birth	3400	23·54	18·73
,,	3100	20·85	16·08
,,	2880	20·65	15·35
,,	3330	16·62	13·12

The figures given here for the maternal blood are low, a capacity of about 19 vol. p.c. being generally accepted for the woman [Peters & van Slyke, 1932; Loewi, 1911]. The values for the fœtal blood are 5–35 p.c. higher than the maternal, and the average found by the authors in thirty newly born children, 21·92 p.c., agrees rather well with the figures given in the table. Nevertheless this average is low, too, if compared with the results collected by Peters & van Slyke who note an oxygen capacity of 32 vol. p.c. for the child during the first day. Considering the haemoglobin standard of the blood of new-born children, an oxygen capacity of 21·92 vol. p.c. may be called low indeed. A hæmoglobin standard of 18–23·7 g./100 c.c. blood, which figures correspond

with an oxygen capacity of 24·12–31·76 vol. p.c., was found by Börner [1928] in twenty-two of thirty children, 25 min. to 14 days old, the highest values being found in the youngest children. The lowest figure found in the thirty children corresponded with a capacity of 20·37 vol. p.c.

In animals, Cohnstein & Zuntz [1884] had obtained quite different results. The maternal blood of the rabbit was proved to contain 15–50 p.c. more hæmoglobin than the fœtal, taken immediately after birth. Under the same conditions, in the dog, the maternal blood contained 25 p.c. more hæmoglobin than the puppy's. In the cavia a difference of 15–20 p.c. was found. The same relation was found in the sheep; here the following figures were obtained:

Sheep	Weight in g.	Hæmoglobin g. in 100 c.c.	O_2 capacity vol. p.c.	Blood taken from
Mother	—	7·3	16·7	Artery
Fœtus No. 1	1290	7·08	14·9	Art. umb.
„ No. 2	1564	6·235	14·1	„

Huggett [1927] used goats for his experiments. From the figures given for a number of pregnant animals an average oxygen capacity of 21·6 vol. p.c. can be calculated, whilst 38 fœtuses at or near full term had an average of 17·5 vol. p.c., the figures varying from 15·5 to 19 p.c. The fœtus of the goat, examined in 1934 by Keys, exhibited a still lower oxygen capacity. In four animals he found 11·7, 12·4, 12·9 and 14·1 p.c. respectively. It must be noted here that these fœtuses were younger than Huggett's; they were still 20–50 days before term.

The results obtained by the various authors are collected in Table I.

TABLE I

	O_2 capacity vol. p.c.		Hæmoglobin g./100 c.c.		
Animal	Maternal blood	Fœtal blood	Maternal blood	Fœtal blood	Author
Sheep	16·7	14·5	7·3	6·66	Cohnstein & Zuntz
Goat	21·6	17·5	—	—	Huggett
„	—	12·8	—	—	Keys
Rabbit	—	—	8·5	6·6	Cohnstein & Zuntz
Dog	—	—	12·32	9·05	„ „
Cavia	—	—	7·55	8·91	„ „
Man	16·1	20	—	—	Haselhorst & Stromberger

When examining these figures a contrast is observed between the animals and man: in the latter the fœtal oxygen capacity is higher than the maternal, whereas in all the animals mentioned a reverse relation is found.

Method

The animals used in our experiments were oxen. The blood of mother and fœtus could be examined in eight experiments, in which the duration of pregnancy varied from 7 to 9 months. The period of gestation is 39–40 weeks in the cow. Maternal and fœtal blood were taken at the same moment, the size of the animal being a favourable circumstance in this regard.

The sequence of events was as follows. After the animal had been put on its left side, the abdominal region was anæsthetized by a 1 p.c. solution of novocain, general narcosis being thus prevented. Then the abdomen was opened near the midline and the gravid horn of the uterus exposed, care being taken that the uterus was not compressed in the abdominal wound. The horn was then incised across a bloodless area, the membranes were cut through and the fœtus was delivered with as few manipulations as possible. All manipulations were performed as quickly as possible after the abdomen had been opened. The fœtus was supported on a wooden grate close by the mother, so that the umbilical cord hung slack without either tension or torsion. The placentæ remained intact and the fœtuses were carefully observed in order to see whether spontaneous respiratory movements were made or not. Fœtal blood was obtained from the art. umbilicalis and from one of the umbilical veins by inserting cannulæ into these vessels. The maternal blood was taken from the art. carotis and the v. jugularis.

In a second series of experiments blood samples were taken from normally born calves immediately after delivery or a few hours afterwards. Here the blood was obtained from the jugular vein. Maternal blood was taken in a similar way and the oxygen capacities of both were compared again.

The measurements of oxygen capacity were carried out with the Barcroft differential manometer. Complete saturation by shaking the blood with air was proved to have taken place within half a minute.

Results

Oxygen capacity of the blood of normal non-pregnant cows. As little is known concerning the oxygen capacity of ox blood, it was measured in eleven normal non-pregnant cows. The results obtained in venous blood are collected in Table II.

Here the average oxygen capacity was 14·55 c.c./100 c.c. blood, the maximum and the minimum being 18·45 and 10·28 vol. p.c. respectively.

TABLE II. Oxygen capacity in normal non-pregnant cows

Animal no.	Date	O_2 capacity (vol. p.c.)
1	25. ii. 35	12·89
2	3. ii. 36	14·45
3	4. ii. 36	12·73
4	4. v. 36	18·45
5	11. v. 36	14·42
6	25. vi. 36	14·85
7	30. vi. 36	15·75
8	3. vii. 36	17·22
9	26. ii. 37	12·58
10	26. ii. 37	14·31
11	18. iii. 37	12·42
	Average	14·55

Fœtal and maternal oxygen capacity. In Table III the oxygen capacity of the fœtal and of the maternal blood is given.

TABLE III. Oxygen capacity of fœtal and maternal blood

Exp.	Date of operation	O_2 capacity		Age of fœtus in months	Blood taken from	
		Fœtus	Mother		Fœtus	Mother
1	6. xii. 34	13·35	12·99	8	Art. umb.	Art. carot.
2	21. xii. 34	12·61	12·00	8	,,	,,
3	21. ii. 35	12·54	11·63	8	,,	,,
4	26. ii. 35	12·15	11·73	7½	V. umb.	,,
5	13. iii. 35	11·35	11·15	8½	—	—
6	26. iii. 35	11·49	11·09	8	Art. umb.	V. jugul.
7	23. iv. 36	15·57	13·87	8	,,	Art. carot.
	Average	12·72	12·07			
8	22. iii. 35	11·68	13·12	8	,,	V. jugul.

As is shown by Table III, the fœtal blood examined in the last months of intra-uterine life has a higher oxygen capacity than the maternal, taken at the same moment. The difference varies from 2 to 12 p.c., the average showing a difference of 5·4 p.c.

The difference between mother and fœtus was found smallest when the fœtus was near full term (Exp. 5). The results under Exp. 8 form an exception to the rule: here the maternal oxygen capacity surpassed the fœtal by 12 p.c. Secondly, the oxygen capacity of the fœtal as well as of the maternal blood was proved to be low in the ox, compared with the values found in the goat, sheep and in man (Table I). The values measured by Keys in the fœtal goat, however, did not differ much from ours.

By comparing the oxygen capacity of the blood of the pregnant cow with that of the non-pregnant animal (Table II) the former is seen to be relatively low again. A difference of not less than 18 p.c. is observed between the average results. Since a similar relation has been

described for the horse but not for the cow, the question arose as to whether the position of the animals during the time that the blood was taken could produce the difference. The normal animals had been on their feet, the pregnant animals had been lying down. Comparison of the blood samples taken in either position in a series of animals proved that a higher oxygen capacity was generally found when the animal was on its feet; the difference is smaller, however, than was found in the experiments, and varies from 3 to 12 p.c. only.

A striking point of difference thus exists between the relation of fœtal and maternal oxygen capacity in the cow on the one hand and in the other animals investigated on the other. Just as had been found in man, the capacity in the cow is generally greater for the fœtal than for the maternal blood. In all other animals investigated (sheep, goat, cavia) a reverse relation had been found.

The oxygen capacity after birth. In some young animals, in which the oxygen dissociation curve was to be studied, the changes of oxygen capacity were followed during the first days after birth. Although it must be kept in mind that in the course of a day differences of 10 p.c. between maximum and minimum quantities of hæmoglobin can occur, perhaps even more, part of the figures given in Table IV show a tendency to a decrease in the oxygen capacity of the circulating blood during the first days after birth. The animals were kept very quiet during the collection of the blood. The calves 2 and 3 were twins, No. 2 being a male, No. 3 a female.

The italicized figures in Table IV suggest that the blood of the newly born animal has a higher oxygen capacity than had been found in another series of experiments for the fœtus (Table III). The averages vary here by 5½ vol. p.c.; they are 18·21 and 12·72 p.c. respectively. (No. 1 in Table IV is excluded since the fœtus was not mature.)

The oxygen capacity of the five mothers (No. 1 is excluded since its oxygen capacity was determined during pregnancy) has an average of 15·3 vol. p.c. immediately after parturition, a figure higher than during pregnancy and higher than that obtained in the non-pregnant animals (14·5 vol. p.c.). It should be noted that the maternal capacity measured during delivery is sometimes found to be higher even than in the new-born animal, although it is not low in the latter. An example of this relation is given by Nos. 2 (and 3) and 6 of Table IV, but it was also found in other cases. So parturition seems to be accompanied by an increase in the oxygen capacity of the maternal blood. Although it was not intended to examine this point we have additional experience which points in the

same direction; a capacity of 13·94 vol. p.c. determined 5½ hr. before parturition, was increased to 15·06 p.c. for the blood taken from the same vein 12 hr. after delivery.

TABLE IV

Animal no.	Sex	Age	O_2 capacity Calf	O_2 capacity Mother
1	♀	Fœtus 8 months. Placenta intact	15·57	13·87
		4 days	16·40	
		8 ,,	13·22	
		14 ,,	12·10	
		20 ,,	14·50	
		23 ,,	15·71	
		29 ,,	14·77	
2	♂	10 min.	*14·85*	15·91
		2 days	14·32	
		4 ,,	14·14	
		8 ,,	14·25	
		14 ,,	11·85	
3	♀	10 min.	*13·89*	15·91
		2 days	12·17	
		4 ,,	11·89	
		8 ,,	12·30	
		14 ,,	8·43	
4	—	3 min.	*20·63*	14·48
		2 days	22·25	
		5 ,,	21·84	
		9 ,,	22·02	
		15 ,,	21·19	
5	—	0 min.	*21·08*	13·99
		1½ days	21·22	
		3½ ,,	22·12	
		5½ ,,	20·95	
		11 ,,	18·44	
6	♀	0 min.	*15·59*	15·84
		1 day	13·84	
		3 days	13·65	
		5 ,,	12·65	
		8 ,,	11·02	
7	♀	1 hr.	*23·41*	16·48
		1 day	21·22	
		3 days	20·09	
		5 ,,	21·14	
		11 ,,	18·82	

B. THE OXYGEN AND THE CARBON DIOXIDE CONTENT OF THE FŒTAL BLOOD

The blood gases in the fœtal and the maternal blood have been measured by Cohnstein & Zuntz [1884] in the sheep, by Huggett [1927], Keys [1934] and Barcroft *et al.* [1934] in the goat.

Haselhorst & Stromberger [1930] determined the blood gases in the new-born child, normally born at full term, before the first respiratory

movement had been performed. The authors found considerable differences for the oxygen, reaching some hundreds p.c.:

	Vol. p.c. O_2		Vol. p.c. CO_2	
	Art. umb.	V. umb.	Art. umb.	V. umb.
Maximum	8·02	14·88	52·85	45·01
Minimum	0·37	5·30	39·60	33·44
Average in 13 children	3·63	10·52	45·81	39·61

The arterial blood, obtained from an umbilical vein, had an average oxygen saturation of 45 vol. p.c. A much smaller divergence of results was observed in children delivered by Cæsarean section under local anæsthesia, i.e. in children who had not been exposed to uterine contractions. Here the oxygen figures are surprisingly low:

	Child				Mother	
	Vol. p.c. O_2		Vol. p.c. CO_2		Vol. p.c. O_2	
	Art. umb.	V. umb.	Art. umb.	V. umb.	Art. epigast.	V. uterin.
Maximum	1·76	5·72	54·12	50·93	16·08	11·83
Minimum	0·22	2·46	40·11	37·75	12·69	7·81
Average of 5 observations	0·84	3·97	47·03	44·95	14·19	10·45

The fœtal arterial blood was saturated to not more than 17–18 p.c.

The results obtained by Cohnstein & Zuntz in one fœtus of the sheep and by Huggett in six goats are collected in Table V.

TABLE V

	Vol. p.c. O_2		O_2 saturation vol. p.c.		Vol. p.c. CO_2		
	Art. umb.	V. umb.	Art. umb.	V. umb.	Art. umb.	V. umb.	Author
Maximum	6·0	12·0	31·6	63·0	54·8	35·0	—
Minimum	0·25	5·0	1·6	27·7	34·8	26·0	Huggett
Average	2·94	7·96	16·3	43·6	41·5	28·9	—
	2·3	6·3	15·4	42·3	47·0	40·5	Cohnstein & Zuntz

Of all animals there was only one, reported by Huggett, in which the oxygen saturation was higher than 50 vol. p.c. Judging from the weight of the fœtus it must have been at or near full term. A somewhat higher degree of saturation (64·1 vol. p.c.) was found by Keys [1934] in fœtuses of the goat about 3 weeks before term.

In a beautiful research made by Barcroft *et al.* [1934] the relation between the age and the degree of oxygen saturation of the blood was

investigated in the fœtus of the goat. Here the saturation was shown to reach a maximum (87 vol. p.c.) 4–5 weeks *ante partum*, viz. when 4/5 of the time of intra-uterine life had elapsed. Then it decreased, especially during the last 2 weeks, and at the moment of parturition the arterial blood was saturated to not more than about 46 vol. p.c.

Method

After the abdomen had been opened under local anæsthesia and the fœtus exposed in the way previously described, samples of fœtal blood were obtained by inserting a cannula in one of the umbilical vessels. It is well known that the umbilical cord of the calf contains two arteries and two veins, each of which is of such diameter that puncture does not offer any difficulty and a rather wide cannula can be used without blocking the circulation in the vessel. The venous blood sample was collected simultaneously with the arterial. Barcroft's differential manometer was used for both oxygen and the carbon dioxide measurement. For the latter the gas was driven out by tartaric acid. As a rule two sets of samples were taken, with an interval of not more than some few minutes. Such duplicate determinations did not differ by more than 5 p.c.

It was impossible to examine all the blood samples immediately. They were all analysed within 24 hr. after they had been taken, most of them within 12 hr. It was therefore necessary to know how far the results of measurement could be influenced by this delay. By some authors the self-reducing activity of the fœtal red blood cells is assumed to be of great importance, and van Slyke's blood-gas pump was therefore preferred to Barcroft's differential manometer in order to prevent the self-reducing activity during measurement.

In a series of experiments blood samples taken from ox fœtuses of various age were therefore kept in the refrigerator for some time under conditions agreeing with those during experiment. At different moments the oxygen content of the samples was measured with the Barcroft differential manometer. The results were as follows. During the first 6 hr. the oxygen content decreased by a maximum of 2 p.c. of the initial value. In some experiments no diminution could be established since the values fell within the limits of exactness of measurement. In the samples kept for 12 hr. the oxygen diminution varied from 1½ to 4 p.c. of the initial measurements, and a decrease of 4–8 p.c. was found in the blood preserved for 24 hr. Thus a correction can be introduced for the values measured so far as this is necessary.

In Table VI the results are collected.

TABLE VI. O_2 and CO_2 in the fœtal blood of the cow

No.	Age of fœtus in months	O_2 Vol. p.c. V. umb.	O_2 Vol. p.c. Art. umb.	O_2 capacity	Saturation p.c. V. umb.	Saturation p.c. Art. umb.	CO_2 Vol. p.c. V. umb.	CO_2 Vol. p.c. Art. umb.
1	7	8·92	2·41	12·15	73·4	19·7	51·57	53·37
		9·07	2·58	—	74·6	20·3	47·77	53·33
2	8	7·48	4·39	11·68	64·0	37·6	54·82	58·10
		7·59	4·36	—	65·0	37·4	55·43	58·84
3	8	6·20	3·46	11·49	54·6	30·4	48·22	50·68
		6·38	3·93	—	56·0	34·4	48·26	49·66
4	8	7·61	2·67	15·57	48·8	18·4	44·60	47·62
		7·43	5·97	—	47·2	38·3	41·88	45·68
5	8½	9·86	5·07	11·35	90·0	46·3	52·57	58·38
		9·79	4·68	—	89·3	44·0	52·71	58·50
	Mother of fœtus 4	Art. carot. 12·40	V. uterin. 8·87	13·87	Art. carot. 89·4	V. uterin. 63·9	Art. carot. 34·77	V. uterin. 40·21

As Table VI shows, the fœtal blood, venous as well as arterial, is saturated to a rather high degree. The figures presented suggest that oxygen saturation reaches its highest value at the end of pregnancy, whereas in the 8th month a condition is found even less favourable than in the 7th month. The results are fairly constant. The number of observations is too small to allow a conclusion concerning the relation between age of fœtus and percentage of oxygen saturation of its blood.

C. THE DISSOCIATION CURVES

One of the most important conditions by which fœtal respiration is governed is the exact form of the fœtal and the maternal oxygen dissociation curve.

Huggett [1927] was the first to give dissociation curves for the fœtal and the maternal blood; he investigated goats at or near full term. A complete series of maternal and fœtal dissociation curves during the period of pregnancy of the goat was given by Barcroft *et al.* [1934]. For man Eastman *et al.* [1933] gave dissociation curves of the maternal blood and of the blood of the child immediately after birth. The same was done some years later by Leibson *et al.* [1936]. Haselhorst & Stromberger had published such curves in 1931. These had not been determined experimentally however, but by calculation using Hill's formula. The relation between K and the hydrogen-ion concentration was determined and it was assumed that Hill's formula with a constant value for "n" could now be applied to both maternal and fœtal blood. In consequence of this procedure the authors obtained curves of the same shape for mother and child. They differed only by position: the fœtal curve was found to the left of the maternal. As will be shown, the procedure followed by these workers is not without objection.

Since the method used for opening the abdomen and the uterus and the

collection of the blood samples has been described previously, some points concerning the dissociation curve only will be given here. Two c.c. of the blood were introduced into a Barcroft saturator of about 290 c.c. content. The saturators were charged with gas mixtures at various partial pressures of oxygen and at a carbon dioxide pressure of about 40 mm. Fifteen min. for equilibration in the 38° water-bath were proved to be sufficient. The blood examinations were made in Barcroft's differential manometer. Measurement of the total gas pressure in the saturator as well as the gas analysis were made in Haldane's apparatus for 10 c.c. Care was taken that not a trace of blood could penetrate into the burette.

The partial carbon dioxide pressure in the saturator was about 40 mm. Hg. As it was kept constant within 2 mm. and the experimentally obtained values are not affected by this difference in a noteworthy degree, a correction will not be applied. At each curve the carbon dioxide pressure will be given. The oxygen pressure was calculated for the temperature of 38° and expressed graphically by plotting it on the abscissa, taking the ordinate for the percentage saturation of the hæmoglobin.

In an analogous way the carbon dioxide dissociation curves have been obtained by varying the carbon dioxide content of the air to which the 2 c.c. of blood in the saturator was exposed.

The dissociation curve of the normal cow. Since the dissociation curve of the normal non-pregnant cow is not known, six healthy non-pregnant animals were used in order to establish it. The blood was taken from the v. jugularis. In Fig. 1 the limits are given of the field within which the curves for the normal animal are found, *i.e.* for the animal which is not pregnant and has not recently been so.

As Fig. 1 shows there is a rather broad area between the limits. Taking an oxygen saturation of 50 p.c. (tension of unloading) the pressure varies between 26 and 37 mm. Hg. This variation is somewhat greater than was found by Barcroft in the normal non-pregnant goat; here the pressure at 50 p.c. saturation varied from 28 to 33 mm. In the region of lower saturation the difference of width of the fields in the two animals is still more conspicuous. Some figures given below show that the area for the goat lies between the limits found for the cow.

Percentage saturation	O_2 pressure (mm. Hg)	
	Cow	Goat
10	7–17	10–12
20	13–25	17–20
40	23–33	25–29
60	30–43	32–37
80	42–57	47–54

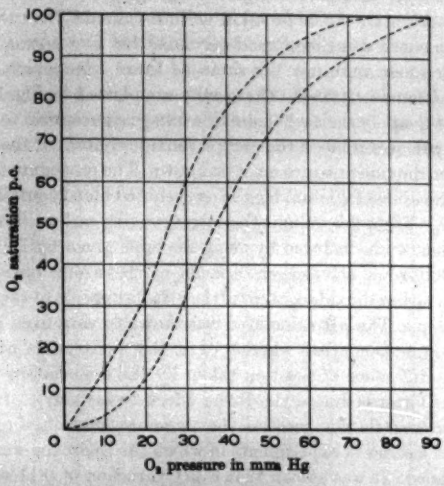

Fig. 1. Limits within which fall the O_2 dissociation curves of the cow, not pregnant and not having been so recently. CO_2 pressure 40–42 mm. Temp. 38° C.

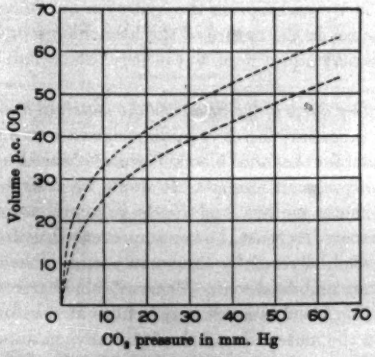

Fig. 2. Limits within which fall the CO_2 dissociation curves of the animals, not pregnant and not having been so recently. The blood is saturated with oxygen. Temp. 38° C.

The relatively broad area suggests that a great variation of hydrogen-ion concentration was to be found in various animals. The carbon dioxide dissociation curve was therefore determined for five normal cows. The animals used here were not the same as those from which the oxygen curves had been determined. The results are plotted in Fig. 2.

In order to apply the carbon dioxide dissociation curves to the calculation of the *p*H of the blood the carbon dioxide content of the plasma at a given carbon dioxide pressure must be known. The procedure followed was this. At a pressure of 40 mm. Hg the oxygenated blood contains 44·5–51·5 vol. p.c. CO_2. From this volume the corresponding carbon dioxide content of the plasma can be deduced by using the table given by Peters & van Slyke [1932], when the oxygen capacity of the various blood samples is known. The carbon dioxide content of the plasma appears to vary from 51·00 to 58·66 vol. p.c. The *p*H calculated was shown to vary from 7·36 to 7·43.

Now the question arises whether or no this variation of *p*H (0·07) can explain the difference of position taken by the dissociation curves, viz. whether the latter is due to the Bohr effect exclusively.

In order to settle this question the oxygen saturation of ox blood was measured in a series of experiments in which the hydrogen-ion concentration was varied. It was shown that a *p*H variation of 0·11 was required to bring about a difference of 10 mm. in the tension of unloading. Since the physiological variation in the *p*H had been found not to exceed 0·07, the wide area between the oxygen dissociation curves of normal cows cannot be explained by variation in the hydrogen-ion concentration alone. Possibly differences in the nature of the hæmoglobin or hæmoglobins in various animals play a part here, but other explanations are conceivable too.

The dissociation curve of the fœtal and the maternal blood. In the adult cow, 8 months pregnant, the oxygen dissociation curve was determined together with that for the fœtal blood. The curve was also established in a great number of pregnant animals. It would be desirable to make this experiment in both mother and fœtus in more animals at various stages of pregnancy. Expense, however, prevented us from doing so.

The fœtus was delivered by Cæsarean section; blood samples were obtained from an umbilical vein. Maternal blood was taken from the carotid artery. The blood samples were defibrinated before examination.

Fig. 3 shows the maternal and the fœtal oxygen dissociation curves. The fœtal curve is found definitely to the left of the maternal and of the area for the normal animals. Starting from zero it rises rapidly and almost rectilinearly to a saturation of about 70 p.c. It then deviates

rather sharply to the right. The curve proves the great oxygen avidity of the fœtal blood. Whereas with the maternal blood a saturation of 50 p.c. requires a pressure of 34·5 mm., a pressure of not more than 11·5 mm. is able to saturate the fœtal blood to the same degree. A difference of oxygen avidity so enormous as appears here has not been found by other investigators either in the goat or in man. In the calf the blood is shown to be saturated to even 80 p.c. at a pressure of not more than 26 mm. Hg.

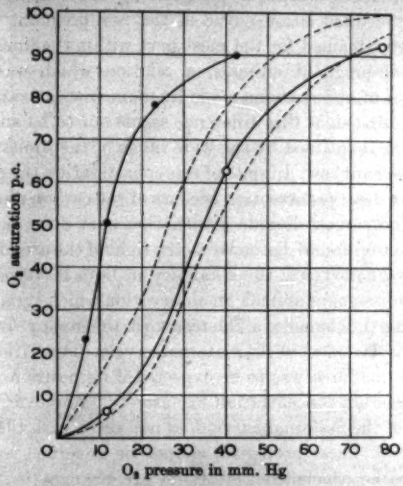

Fig. 3. Cow pregnant about 8 months. (o) Maternal blood, (•) fœtal blood. CO_2 pressure 43–45 mm. Hg. Temp. 38·5° C.

Huggett had found that the fœtal blood was even less saturated with oxygen than the maternal at a given oxygen pressure. Another result was obtained by Barcroft *et al.* [1934] who found the same relation between the fœtal and maternal curves as ourselves. Also Leibson *et al.* [1936] found the curve of the child immediately after birth to the left of the maternal. The physiological consequences in the cow are far more intensive however than in the goat and in man. The difference is shown clearly by some figures given in Table VII.

A second point of difference is this. The maternal curve found by Barcroft *et al.* during the latter half of the period of pregnancy was displaced to the right of the normal field. Leibson's maternal curve also,

TABLE VII

			O_2 pressure (mm. Hg)		
			Mother	Fœtus	Author
Goat:	Fœtus, 19½–20½ weeks	Saturation 50 p.c.	35	22	Barcroft *et al.*
Man:	Child normally born	,,	32	25	Leibson *et al.*
Cow:	Fœtus about 8 months	,,	34·5	11·5	Roos & Romijn

determined at the moment of parturition, was slightly displaced to the right of the dissociation curve of the non-pregnant woman. Our maternal curve remained for the most part within the limits determined for normal non-pregnant animals, a relation which was affirmed by a great number of observations made by us in cows at various stages of pregnancy. At first sight this difference seems not to be an essential one since it might be explained by the wide range of the limits of the curves for the non-pregnant cow. In spite of this consideration the position of the maternal curve deserves attention because of the carbon dioxide dissociation curve. The carbon dioxide dissociation curves for both fœtal and maternal blood were found distinctly to the right of the area determined for normal non-pregnant cows: the alkalinity in both is considerably lower than in the non-pregnant animal, an observation which agrees with what is known regarding the change of alkali reserve in pregnancy. In consequence of this fact a displacement of the maternal oxygen dissociation curve to the right of the normal area was to be expected if its position was governed by the hydrogen-ion concentration exclusively. Moreover, the more inflected shape of the maternal curve does not agree with that of the non-pregnant animal. These properties cannot be governed by the *p*H. It must therefore be concluded that there are changes in the maternal blood other than an increase of hydrogen-ion concentration, produced by the condition of pregnancy. Among these a change in the properties of hæmoglobin may be considered as taking a first place.

The maternal oxygen dissociation curve during pregnancy will not be further discussed here. The subject has been treated in another paper [1937].

A striking occurrence is the coincidence of the maternal and fœtal carbon dioxide dissociation curves, shown by Fig. 4. In spite of this, a wide discrepancy between the oxygen curves had been found (Fig. 3) involving both position and shape of the curves. The discrepancy is still more conspicuous in the logarithmic curves (Fig. 5). They are obtained from the same measurements as are those in Fig. 3, and in this Hill's equation is used in its logarithmic form.

It is clear that in Fig. 5 Hill's exponent (n) is indicated by the tangent of the angle which the curve forms with the x-axis. By calculation n is found to be 2·81 for the maternal and 1·80 for the fœtal blood. It follows from this that the oxygen dissociation curves given by Haselhorst & Stromberger cannot be exact since these authors assumed that the same value for n could be used for the construction of both fœtal and maternal curves.

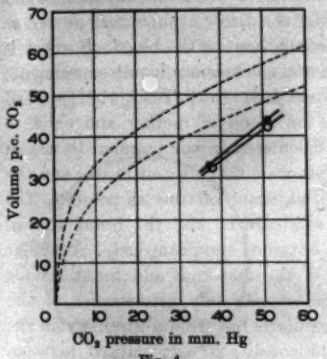

Fig. 4.

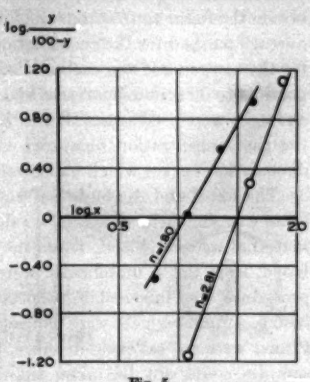

Fig. 5.

Fig. 4. CO_2 dissociation curves of maternal (o) and fœtal blood (•). The dotted lines indicate the limits of the CO_2 dissociation curves for normal non-pregnant cows. Blood saturated with O_2. Temp. 38° C.

Fig. 5. O_2 dissociation curve (see Fig. 3) in logarithmic form. Maternal blood (o) and fœtal blood (•). Other conditions as in Fig. 3.

The coincidence of maternal and fœtal carbon dioxide dissociation curves furnishes evidence again that the divergence of position and shape between fœtal and maternal oxygen dissociation curves cannot be accounted for by changes in the reaction of the blood, but that differences between the properties of hæmoglobin of mother and fœtus are the probable cause.

D. The tension of the gases in the fœtal and the maternal blood

The tensions of the gases in the fœtal and the maternal blood were determined indirectly, viz. by interpolating in the dissociation curves the percentage of gas saturation found in the blood samples of the animals.

Experimental determination of the gas pressures in the blood on the fœtal and the maternal side of the placenta in one and the same pregnant animal has not yet been carried out. As early as 1877 Zuntz made an estimate of the difference of oxygen pressure between maternal and fœtal blood in the rabbit. Huggett [1927] was the first to give figures for the blood-gas tensions of fœtus and mother, taking the goat at or near full term. Owing to technical difficulty it was not possible, however, to obtain the fœtal figures from one fœtus. He had recourse, therefore, to an average method for the construction of the dissociation curves as well as for the oxygen and the carbon dioxide content of the blood. Nor was it possible to determine maternal and fœtal gas tensions in one experiment; averages were used again. Haselhorst & Stromberger [1931] applied the gas concentration measured in the blood of mother and child to dissociation curves which had been calculated in the manner discussed.

The fœtal and the maternal bloods were taken by us in one and the same experiment and within as short a space of time as possible. The maternal arterial blood, from the art. carotis, and the fœtal arterial blood, from the v. umbilicalis, were obtained simultaneously. A similar procedure was followed in respect of the maternal and fœtal venous blood samples which were collected immediately afterwards. If the former were not taken from the v. jugularis but from a uterine vein the samples could not be taken simultaneously; 20 sec. elapsed between the collections.

Measurement of the content of gases in the fœtal blood was made within a few hours. No determination was made more than 5 hr. after the sample had been obtained.

In Table VIII some figures are collected.

TABLE VIII. Cow pregnant about 8 months.
Fœtus delivered by Cæsarean section

Time	Blood taken from (*M* = mother)	O_2 Vol. p.c.	O_2 Saturation p.c.	O_2 Pressure mm. Hg.	CO_2 Vol. p.c.	CO_2 Pressure mm. Hg
10·07	Art. umb.	2·87	18·4	5·5	47·62	50
	V. umb.	7·61	48·8	11·5	44·60	48
10·10	V. umb.	7·43	47·7	11·0	41·88	46
	Art. carot. (*M*)	12·40	90·0	70·0	34·77	41
10·15	Art. umb.	5·97	43·0	10·5	45·68	49
	V. jugul. (*M*)	7·20	52·0	36·0	45·68	52
10·24	V. uterin. (*M*)	9·83	71·0	45·5	34·43	39
10·31	V. uterin. (*M*)	7·92	57·0	37·5	46·00	54
	V. jugul. (*M*)	7·98	58·0	38·5	38·06	42

The figures given in the last column of Table VIII demand comment. Since they are deduced from dissociation curves for blood saturated with oxygen they have been corrected because of the fact that blood with reduced hæmoglobin is able to take up more carbon dioxide than blood oxygenated to 100 p.c. The figures from Table VIII are used in the scheme given in Fig. 6.

Fig. 6 shows that the difference of pressure, by which oxygen is caused to flow from the arterial maternal to the fœtal side of the placenta, is not less than 64 mm., whilst for the carbon dioxide transport from the fœtal to the maternal side a difference of pressure of 9 mm. is available. The carbon dioxide pressure in the fœtal arterial blood, which leaves the placenta by the umbilical veins, is still higher than in the venous maternal

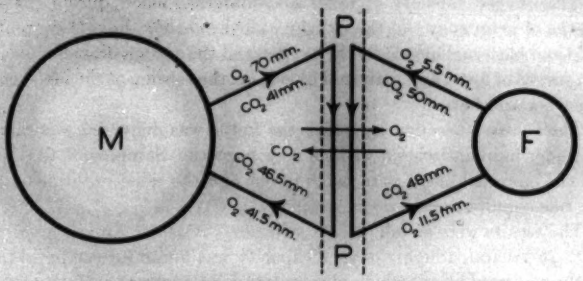

Fig. 6. Gas tensions in the fœtal and the maternal blood.
(*M*) = mother, (*F*) = fœtus, (*P*) = placenta.

blood. On the other hand the maternal venous blood contains oxygen under still higher tension than the fœtal arterial blood. If the direction in which fœtal blood passes the maternal should be reversed, the differences of pressure between the blood gases found on either side of the placenta still remain considerable. Consequently the process of exchange of oxygen as well as of carbon dioxide may be expected to proceed as long as maternal and fœtal bloods pass each other.

The figures given in Fig. 6 are only approximations. This applies especially to the blood taken from one of the branches of a uterine vein, since this is not pure placental blood but a mixture of blood coming from the cotyledons and of blood by which the uterine wall has been transfused. Pure cotyledon blood, however, cannot be obtained. In Fig. 6 the average value was therefore taken of the results obtained in blood samples from two uterine veins. The oxygen tension of the blood which leaves the

maternal placenta, if measured before the uterine wall is transfused will probably be higher, the carbon dioxide tension lower.

The question to what extent differences in gas pressure on either side of the placenta are able to ensure fœtal respiration by diffusion cannot be discussed before the diffusion constants of placental tissue for both oxygen and carbon dioxide have been determined. Besides this, other quantitative figures are needed. The differences of pressure of the blood gases are, however, shown to be considerable.

Summary

The following conditions of fœtal respiration were studied in the cow. (*a*) The oxygen capacity of fœtal and maternal blood during the last months of pregnancy; (*b*) the oxygen and the carbon dioxide content of fœtal and maternal blood; (*c*) the oxygen and the carbon dioxide dissociation curves of fœtal and maternal blood; (*d*) the tension of the blood gases in mother and fœtus.

The Cæsarean section by which the fœtus was delivered was carried out under local anæsthesia without any narcotic. Samples of the fœtal and the maternal blood were taken in one and the same experiment and as a rule simultaneously.

The results were as follows.

1. In general, a higher oxygen capacity was found for the fœtal than for the maternal blood, which observation is in contrast with the results in other animals and in agreement with experience gained in man.

2. During the first hours after parturition the oxygen capacity of the maternal blood seems to rise, so that it may surpass the capacity of the blood of the new-born animal, which in its turn is higher than that during fœtal life.

3. The degree of oxygen saturation measured in the fœtal blood during the last 2 months of intra-uterine life was higher than had been described for the human fœtus at birth and for the sheep and the goat at corresponding moments. Some weeks before term the arterial blood was found saturated to about 90 p.c., the venous blood to 45 p.c.

4. After the limits of the oxygen dissociation curves for the blood of normal non-pregnant cows had been established, the curves found for pregnant animals were shown to fall for the most part within these limits.

5. The oxygen dissociation curve of the fœtus was found to the left of the curve of its mother and of the area established for the normal animals. It differs from the maternal curve by shape and position. The

fœtal curve shows the great oxygen avidity of the fœtal blood, in consequence of which a pressure of 11·5 mm. Hg is already able to saturate the fœtal blood to 50 p.c., a saturation, which for the maternal blood requires a pressure of 34·5 mm.

6. From a study of the carbon dioxide dissociation curves evidence was obtained that shape and position of the fœtal oxygen dissociation curve cannot be accounted for by change in hydrogen-ion concentration. It must be attributed to a difference between the properties of fœtal and maternal hæmoglobin.

7. Measurement of the tension of gases in the fœtal and in the maternal blood revealed a difference of partial pressure on either side of the placenta of 64 mm. for the oxygen and 9 mm. for the carbon dioxide. The maternal blood, which leaves the placenta by the uterine veins, has still a higher oxygen pressure and a lower carbon dioxide pressure than the fœtal arterial blood returning to the fœtus through the umbilical veins.

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THE ELECTROCARDIOGRAM OF THE GUINEA-PIG

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(*Received* 5 *January* 1938)

THIS investigation has dealt with the electrocardiograms of normal, apparently healthy guinea-pigs living under laboratory conditions. It formed part of the observations carried out upon animals which were later subjected to the action of various toxins. Records were obtained from fifty-seven guinea-pigs, and in every case a second record was made, at an interval of 1–4 weeks after the first. A few animals were examined daily for 1 week. Electrocardiograms taken on different occasions from any one animal were nearly always identical; such variations as did occur were of rhythm alone. No anæsthetics were used.

TECHNIQUE

The animal was suspended from two metal stands. A half-inch strip of adhesive plaster was passed cephalad to the right shoulder, across the thorax and caudad to the left shoulder. A second strip was passed cephalad to the left shoulder and caudad to the right, all four ends being brought together above the scapulæ in the mid-line of the body and seized by a small clamp carried on a glass rod by a metal stand. The posterior part of the animal was similarly supported by a single strip of adhesive plaster 1 in. wide passing beneath the belly. The plaster helped to restrain the animal, as well as support it, by adhering firmly to the hairy coat. A little depilation occurred removing the plaster. To discourage wriggling, which might shake the electrodes, the guinea-pig's head was enclosed in a calico bag, having small vents in it. This hood was held in position by a purse-string of india-rubber. When the hood had been placed in position, saline-sodden darning wool, four strands thick, was wound round each fore-paw and the left hind-paw. The paws

were then covered by stout pliable rubber "boots" or finger-stalls provided with rubber side-tubes, through which were brought out the ends of the wool strands. The ends of the tubes were then placed in three porcelain pots containing saline, into which dipped the electrodes leading off to the amplifier. The "boots" and their side-tubes prevented short-circuiting when the animal's feet touched one another, and also prevented the moistened wool from drying, a constant source of trouble when dealing with animals, such as mice, too small to wear such "boots".

The recording system consisted of a four-stage, resistance-capacity coupled amplifying unit, operating a Matthews oscillograph. The records were not standardized, but the degree of amplification was kept constant.

The convention adopted was that a negative variation at the grid of the first valve produced an upward deflexion of the oscillograph mirror. In lead I, the right fore-paw was led off to the grid, the left being "earthed". In lead II, the left hind-paw was "earthed", whilst the right fore-paw remained connected to the grid. For lead III, the left fore-paw was led off to the grid and the left hind-paw to earth. In this way records were obtained agreeing with the usual technique of the string galvanometer.

Results

The essential time-relations of the electrocardiogram are given in Table I. The most striking feature of these is the unexpected degree of variation of the *PQ* interval.

Table I. Time relations of the guinea-pig electrocardiogram

	Mean	Maximum	Minimum
P-wave	0·016 sec.	0·025 sec.	0·008 sec.
PQ interval	0·036 ,,	0·055 ,,	0·024 ,,
QRS complex	0·013 ,,	0·021 ,,	0·008 ,,
ST interval	0·059 ,,	0·084 ,,	0·041 ,,
T-wave	0·022 ,,	0·034 ,,	0·013 ,,
Systole	0·095 ,,	0·121 ,,	0·072 ,,
Cardiac cycle	0·183 ,,	0·288 ,,	0·162 ,,
Ratio systole/cycle	0·52	0·59	0·41
Frequency	327 per min.*	400 per min.	232 per min.

* Benedict [1915] gives 267 per min. Koenigsfeld & Oppenheimer [1922] and Schott [1920] both gives 290 per min. According to Buchanan [1910] the frequency is 300 per min.

Fig. 1 shows three typical electrocardiograms. Each part of the *PQRST* complex is subject to considerable variations.

The *P*-wave is generally, but not always, well marked. When present, it is always directed upwards. When it does not appear in lead I, then its height in lead II is the same as that in lead III, whilst if it be absent

from lead III, it is of the same height in lead I as in lead II. It is never missing from lead II. In its commonest form it has blunt, notched or plateau appearance. Sometimes its apex is sharp. On six occasions it has been so deeply notched as to appear bifid.

The *QRS* complex forms the most constant part of the record. The main deflexion is always upwards. In leads I and II the whole complex is usually represented by a single monophasic deflexion, *Q* and *S* being

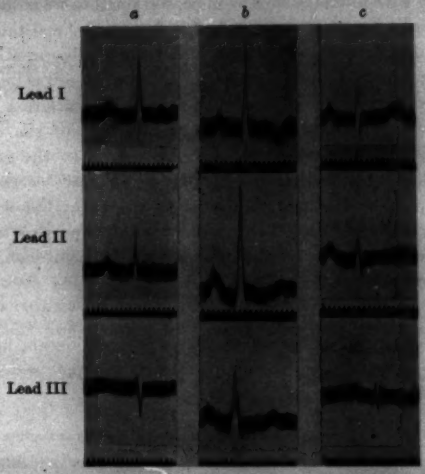

Fig. 1. Types of guinea-pig electrocardiogram. Note: (*a*) distinct left-sided preponderance and bifid *P* in lead I; (*b*) inverted *T* in lead I; (*c*) small amplitude of deflexions and double swing of electrical axis during inscription of *QRS*. Time in 1/100 sec.

absent. In lead III the complex may be monophasic, diphasic or triphasic. The monophasic variety is upright, whilst in the other two types the first deflexion is upright.

The electrical axis of the heart was calculated in fifty records which satisfied the requirements laid down by Einthoven [1906]. In these records the electrical axis at the moment when the *R*-wave was being inscribed varied from 0 to 76°; 80 p.c. lying between 20 and 60° and 50 p.c. between 30 and 45°. In addition to these wide limits in the initial "set" of the electrical axis, there is considerable variation in the average pathway pursued by the excitatory process over the ventricles and hence in the degree and direction of the "swing" of the axis.

The *T*-wave is generally directed downwards in lead I, but is always upwards in leads II and III.

Although the rhythm of the guinea-pig's heart is generally very regular, on five occasions records have been obtained showing interpolated ventricular extrasystoles, and occasionally the rhythmicity of the pacemaker itself is disturbed. Three records showed an oscillation of the isoelectric line coinciding with the respiratory frequency (Fig. 2).

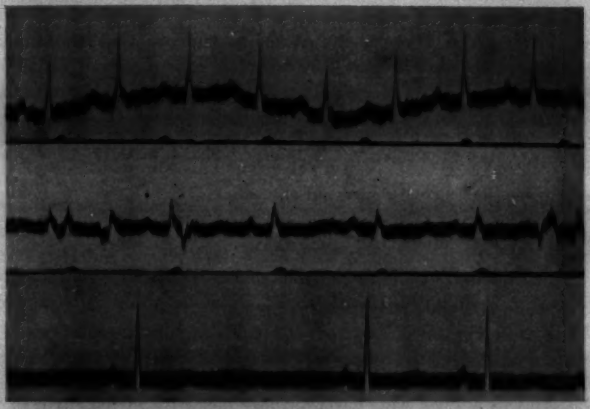

Fig. 2. Types of cardiac arrhythmia in the guinea-pig, showing (*a*) respiratory rhythm, (*b*) ventricular extrasystoles, and (*c*) "dropped" nodal beat. Time in 1/5 sec.

Discussion

The use of electrocardiography as an indication of the effect upon the beat of any experimental interference with the animal necessitates a knowledge of the limits of variation of the normal characteristics of the electrocardiogram. The attempt is made in this paper to supply that information as far as the guinea-pig is concerned.

The most striking feature of the results obtained is the degree of variation, both qualitative and quantitative, in the normal picture. Apart from its invariable occurrence in lead II, there is nothing constant in the occurrence or shape of the *P*-wave. The conformity of the *QRS* complex to a standard shape is only very approximate and, especially in lead III, subject to numerous exceptions. The angle of the electrical axis when *R* is being inscribed differed by as much as 76° in different

animals. In lead I the *T*-wave is more often than not inverted. Thus it is impossible to give a standard description of the normal electrocardiogram in the guinea-pig that would cover all, or even a majority, of cases. On the other hand, any appearance in the electrocardiogram of a given animal at one time is always reproduced exactly (with the occasional exception of variations in rhythm) on a subsequent occasion, provided there has been no interference with the action of the heart.

The range of variation of the *P-Q* interval, in particular, is very great when compared with the constancy in length of this interval in the human electrocardiogram. There is no reason to suppose that the animals, living under ordinary laboratory conditions, suffered from cardiac disabilities evidenced by varying degrees of heart-block. These animals were afterwards used for a study of the cardiac effects of various toxins and some of them did develop a true prolongation of the *P-Q* interval, that is, an increase not only over the original measurement in each case, but beyond the limiting figures for the series.

Summary

1. A method of obtaining the electrocardiograms of conscious guinea-pigs is described, involving a minimum of restraint and discomfort.

2. The characteristics of the electrocardiogram of the guinea-pig are presented and discussed.

3. In this animal, wide variations are met with in the shape of the deflexions and their time relations.

4. The guinea-pig's heart is especially subject to variations in the initiation and conduction of the excitatory process.

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ANTIBODIES ORGAN-SPECIFIC AGAINST THE ANTERIOR BODY OF THE PITUITARY GLAND

By OTTO KESTNER

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(*Received* 20 *January* 1938)

Last year I published an investigation on antibodies organ-specific against the thyroid gland [1937]. I treated sheep with intravenous injections of extracts of the thyroid glands of pigs and injected the serum of the sheep into rabbits. After some weeks the basal metabolic rate of these rabbits was lowered. I have observed that the metabolic rate stays at this lowered level for a year. In this paper similar experiments with organ antibodies specific against the anterior body of the pituitary gland are reported.

The literature is not discussed, because my method is a new one.

Observations in the physiological laboratory in Hamburg had shown, that specific dynamic action (s.d.a.) depends upon the anterior body [Kestner, 1928; Nothhaas & Never, 1930]. I have now determined the s.d.a. in rats before and after injection of an antipituitary serum. The anterior body of oxen was roughly minced and washed thoroughly with Ringer's solution, the residue frozen, finely ground with sand and dissolved in Ringer's solution. A milky solution containing about 60 p.c. of the nitrogen of the gland and only traces of blood was obtained.

Methods

Rabbits were injected several times with this extract, first intravenously, and later subcutaneously. Four rats were injected four times with 3·5–4 c.c. of the serum of these rabbits on 9, 11, 29 and 30 October. One of these four rats died by accident.

Basal metabolic rate of, the s.d.a. in, the three injected rats were determined before the first injection and at the beginning of December. For the basal metabolism the rats were starved about 24 hr.; for the s.d.a. they received 10 g. raw meat, and the metabolic rate was determined during the second and third hour after feeding; all experiments

were made only during the forenoon and the first hours of the afternoon. I used for the metabolism determinations my apparatus described in 1910 [see also Gröbbels, 1934], in which CO_2 is absorbed, but not determined. The temperature was 28–29° C. and the readings—every 10 min.—were made only during complete rest of the rats. In all experiments a series of readings was made. The surface area was reckoned by Benedict's method with $k = 9{\cdot}1$.

Results

Table I

		Before injection		After injection	
Rat no.	Wt. g.	Basal cal.	s.d.a. p.c.	Basal cal.	s.d.a. p.c.
0470	287	668	58	639	14
0404	280	678	38	674	12
0405	319	707	34	716	10

Two other rats had been injected at the same time with serum of another rabbit, not immunized against pituitary, and the metabolic rates were determined at the same time as those of the three rats. The results are given in Table II.

Table II

		After injection	
Rat no.	Wt. g.	Basal cal.	s.d.a. p.c.
0509	256	700	32
1320	241	696	38

Two rats were not injected, and the results in these are given in Table III.

Table III

Rat no.	Wt. g.	Basal cal.	s.d.a. p.c.
0472	255	683	40
0403	271	690	33

The differences in the basal metabolic rate before and after the injection were 0–1 c.c. as measured on the burette of the apparatus; the differences in the s.d.a. before and after injection were 10–15 c.c.

The number of the experiments is small, but I think that they are sufficient to show that the specific dynamic action is lowered by the treatment with the serum organ specific against the anterior body of the pituitary gland. The three rats are still living and the other functions that are dependent upon the pituitary gland will be investigated later.

SUMMARY

The specific dynamic action in rats is considerably lowered by a serum organ-specific against the anterior body of the pituitary gland.

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THE INTERPRETATION OF POTENTIAL CHANGES IN THE SPINAL CORD

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The first records of electrical activity in the spinal cord made by Gotch & Horsley [1891] showed that potential changes did accompany reflex activity, but only recently have these been examined in detail and correlated with reflex phenomena. In the last five years much work has been published on the action potentials of the spinal cord [Gasser & Graham, 1933; Hughes & Gasser, 1934*a*, *b*; Umrath & Umrath, 1934; Barron & Matthews, 1936*a*, *b*; Eccles & Pritchard, 1937; Hughes *et al.* 1937; Matthews, 1937]. Several of these authors have shown that spike potentials recorded from the spinal cord may be attributed in part at least to the conduction of impulses along axones of the cord, and as might be anticipated from the complex nature of the pathways which these traverse, they are not as simple as the action potentials of peripheral nerve, but there is nothing to suggest that these action potentials arise from processes different in nature from the transient depolarization of fibres characteristic of peripheral conduction. In addition to the spike potentials all authors have found much slower electrical changes which show a correlation with the reflex activities which they accompany.

All the observations referred to above except those of Umrath, Eccles, and ourselves, were made by recording from two electrodes placed on the surface of the exposed spinal cord. The changes observed are often extremely complex, for not only are the activities producing them complex, but they may also be occurring in several sites with different orientations relative to the electrodes. This may lead to the appearance of potentials of opposite sign when the same process occurs in different positions.

In the course of work on the impulses of dorsal roots [1935] we frequently observed slow potential changes occurring in the roots when

neither lead was touching the spinal cord. At first it seemed these might be due to summated impulse activity, but later work showed this view to be untenable. These slow potential changes were in fact arising in the spinal cord and spreading along the dorsal roots by a mechanism apparently identical with the electrotonus by which an applied potential spreads along a stretch of peripheral nerve [Barron & Matthews, 1936*a*]. The present paper shows that the potential changes in the dorsal roots give an indication of changes occurring in the regions where the fibres of which they are composed terminate. Similarly, the potential changes in the ventral roots give evidence of the changes occurring in the neighbourhood of the ventral horn cells, and, as we have reported [Barron & Matthews, 1936*b*], the discharge of impulses by the motor neurones is very closely associated with these potential changes which spread along the ventral root. The association of slow potential changes with the discharge of impulses by neurones shown to occur in isolated ganglion systems by Adrian [1931] and by Eccles [1936] also occurs in motor neurones of the spinal cord.

Observation of the electrical changes of the spinal cord *via* the changes in the roots has important advantages over direct observation from the surface of the cord: the leads are more specific and only give certain components of the cord potentials, the complex polyphasic spike potentials arising from impulse conduction in the cord are eliminated or become very small, the records obtained are of a relatively simple nature facilitating their analysis, and the leads are anatomically localized.

The present paper is a description of the nature and occurrence of these electrotonic potentials of the roots, and in addition we attempt to correlate them with cord functions, and with the observations of other authors who have led direct from the surface of the cord. Our experiments have been made on cats, frogs and a few monkeys. The similarities in the results from these animals are close enough to justify the assumption that processes observed are the same, and are the result of the common phenomena of synaptic action. We have been reluctant to use electrical stimulation, and have, wherever possible, made our deductions from the effects of natural stimulation *via* sensory nerve endings. Any volley of impulses evoked by electrical stimulation of a mixed nerve trunk or root presents to the cord an impulse pattern which is temporally, spatially, and functionally abnormal, for fibres of diverse function are thrown into action simultaneously. Even one of the most synchronous sensory volleys occurring naturally, that resulting from a tendon tap, is much more asynchronous than that resulting from

electrical stimulation. Moreover, it is travelling in fibres of associated function, those which end peripherally in stretch receptors and belong to the A group of Gasser & Erlanger [1927]. While volleys evoked electrically are of great value in deducing the nature of the processes involved, they do not necessarily show how the mechanisms work in the intact animal. We have therefore, wherever possible, checked results from electrical stimulation by natural sensory stimulation.

Methods

Our experiments were carried out on frogs, cats, and a few monkeys. In all cases the animals were anæsthetized with ether, the spinal cord was exposed in the lumbar region, and then decerebration was effected through a trephine hole (by section of the brain stem in frogs), and the anæsthetic discontinued (in many cases the cord was sectioned in the mid-dorsal region). The roots for examination and any peripheral nerves required were prepared and the animal set up in a screened box which could be warmed electrically and the air in it moistened from a heated water-tank. In some cases subcutaneous injections of curare insufficient to immobilize the respiratory muscles, but sufficient to stop limb movement were given.

The potentials were amplified with a direct coupled amplifier previously described [Matthews, 1935] and recorded with a moving iron oscillograph [Matthews, 1929]. Occasionally condenser coupling (4μF., 1 megohm leak) was introduced between the first and second stages to obviate drift, and the direct coupling used to confirm observations made with condenser coupling in use. A balanced input stage [Matthews, 1934] was used, and recently a modification of this, for which we are indebted to Dr Tonnies (verbal communication), has been employed in which a common cathode resistance is used for the paired valves. This makes the amplifier very insensitive to changes in the potential of the grids relative to earth. The animal may be earthed and yet the potentials recorded remain those occurring between the two electrodes connected to the grids. Bromide paper cameras were used to record the oscillograph deflexions, signals and the time-marker.

A double neon lamp stimulator [Matthews, 1935] was used for electrical stimulation. The rate and strength of stimulation applied to two different nerves could be varied independently. Two coreless induction coils were also employed; the primary circuits could be broken by a Lucas pendulum to give two stimuli at short predetermined intervals.

Part I. Potential changes in the dorsal roots

If a small lumbar dorsal root be sectioned and the central end placed on recording electrodes, the proximal one being about 2 mm. from the cord, any form of sensory stimulation in the hindquarters results in the appearance of slow potential fluctuations. Whole roots were usually used in frogs and small fibre bundles in cats; to avoid repetition we hereafter use the terms root or rootlet to refer to whole or part of a segmental supply. In Fig. 1 A, B are shown the changes occurring thus

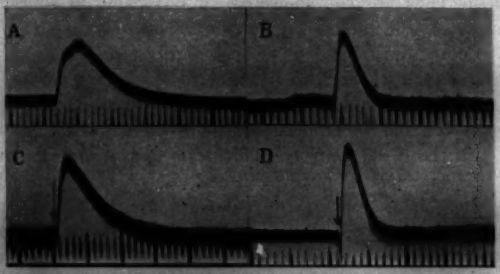

Fig. 1. Potential changes occurring in the central portion of a cut dorsal rootlet. Proximal electrode in each case 2 mm. and distal 10 mm. approx. from point at which root enters the cord. Upward deflexion indicates negativity of proximal electrode. A, frog, 16° C., stimulus light tap on ipsilateral toes. B, cat, ditto. C, frog, 16° C., stimulus single break shock to adjacent rootlet. D, cat, ditto. (Time marker shows 0·1 and 0·02 sec. intervals.)

This and all subsequent records read left to right, are in linear co-ordinates, and about one-third of the size of the originals. The escape of stimulus is marked by an arrow in records where electrical stimulation was used. All records unless otherwise stated taken with direct coupled amplifier and throughout upward deflexion = negativity of lead nearest the cord.

in a frog and a cat when a brief asynchronous volley of sensory impulses reaches the cord. The potential change has a duration of about $\frac{1}{10}$ sec. in the cat and $\frac{1}{4}$ sec. in the frog (16° C.). The electrode nearest the cord becomes negative relative to the distal electrode. In Fig. 1 C, D are shown the potential changes evoked by a single maximal shock to a peripheral nerve. It will be seen that the potential develops rapidly after a short latency (2–10 msec. in the frog and 1–4 msec. in the cat), that the potential changes have a duration of about $\frac{1}{4}$ and $\frac{1}{10}$ sec., and resemble those evoked by natural stimulation (Fig. 1 A, B).

Position of electrodes

As we have already reported [1936*a*], these potentials cannot be summated nerve action potentials, for their size decreases as the electrodes are moved away from the spinal cord. They are reduced by about half for every 1·4 mm. (in the cat) and 1·7 mm. (in the frog), through which the proximal electrode is moved away from the spinal cord. The occurrence of these potentials in the root is not due to simple electrical spread from the spinal cord, for they vanish when the root is crushed, or is replaced by a moist thread touching the cord at the point of entry of the root. They are not abolished by novocaine in concentrations sufficient to abolish impulse conduction. They only appear when the nerve is in normal physical, but not necessarily physiological condition. It thus appears that changes occurring in the spinal cord produce electrotonus in the fibres terminating therein, which spreads to their extracordal portions and may be observed there without the leads being in contact with the cord itself. The potentials thus recorded give an indication of events in the spinal cord, and, as will be shown below, the roots appear to act as specific leads to particular parts of the grey matter.

The largest potentials are recorded when one electrode is on a cut rootlet and the other is touching the cord at the point where the root enters it (the approximate maxima are 5 mV. in frogs and 1·5 mV. in cats; these maxima are only seen if the root is dissected clear up to its point of entry to the cord). If both leads are on the cord a volley of impulses is followed by a polyphasic spike potential and a complex slow wave as has been shown by Gasser & Graham [1933]. The slow wave recorded from the surface of the spinal cord is only about one-fifth the maximum amplitude seen with one lead on the cut rootlet and the other touching the rootlet where it enters the cord, and it never has the simple form that is observed when both leads are on the rootlet. When the lead electrodes subtend part of the cord large spike potentials appear, and the slow wave is complex, whereas led from a root only, it appears as a simple negativity of constant shape. The significance of this will be discussed later.

Relation of the slow potential to the entry of impulses

In the cases considered above the recording leads were on a severed rootlet, and therefore, impulses could only enter by other roots. If a maximal volley is sent in by the same rootlet on which the recording electrodes are placed, the spike potential of the centripetal volley is

followed by a prolonged negativity in the same way as when the volley enters by neighbouring roots and it is of the same magnitude. This is illustrated in Fig. 2. The negativity follows on from the action potential of the centripetal volley. This is perhaps clearer in Fig. 2 C, D, where the volleys are evoked by a tap on the skin of the foot and in place of the single maximal action current the asynchronous action currents of the sensory impulses entering the cord can be seen followed by the evolution of the central negativity.

Fig. 2. Potential changes in an intact dorsal rootlet when impulses reach the cord *via* its fibres. A, frog, 16° C., stimulus a single shock applied to the ipselateral sciatic nerve, recording electrodes 1·5 and 12 mm. from point where root enters cord. All other roots cut. (Earth-free amplifier.) B, cat, shock to rootlet *via* electrodes 3 cm. below recording electrodes which were 1 and 9 mm. from cord. C, as A, stimulus tap on skin of foot. D, as B, ditto. (Time marker shows 0·1 and 0·02 sec. intervals.)

If no impulses enter by the root observed but only by neighbouring roots the potential is of the same size or only slightly smaller than when impulses also arrive by the root observed, so it is clear that the effect is able to spread within the cord. The potential change may even be detected in roots five or six segments from the point of entry of a volley, but the slow negativity becomes smaller as the distance between roots of entry and that of observation is increased, and is smaller for the same separation when the root observed lies caudal to that of entry than when it is cephalad. The question thus arises whether this spread of effect to a large area is due to simple electrical conduction or to nervous conduction within the cord. This question is answered by Fig. 3, which shows the potentials evoked in a severed dorsal root when a volley enters by (A, C) and ipselateral root, and (B, D) by a contralateral root, both of the segments caudal to the recording electrodes in (A, B) cat and (C, D)

frog. It will be seen that the potentials are somewhat similar but that the latency and duration of that evoked by the contralateral volley is longer than that evoked by the ipselateral volley. If the potential evoked by the contralateral volley were affecting the root under observation by electrical spread the two would be identical. The differences can only be accounted for if the spread occurs by a nervous process.

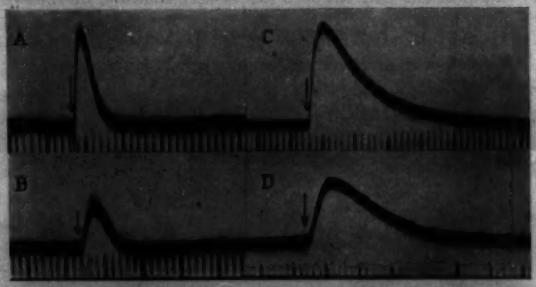

Fig. 3. Dorsal root potentials: A, B, cat, electrodes 2·5 and 12 mm. from cord on root sectioned peripherally: A, stimulus break shock to adjacent rootlet ipselateral; B, ditto contralateral. C, D, frog, 11° C., electrodes 2 and 11 mm. from cord: A, stimulus a shock to the adjacent ipselateral root; B, contralateral ditto. (Time marker shows 0·1 and 0·02 sec. intervals.)

The delay of the effect after a contralateral stimulus shows that the change occurring earlier on the opposite side of the cord does not spread electrically and affect the electrodes, although in the frog's cord the distance between the dorsal horn of the opposite side and the root on which the electrodes are placed is very small indeed. This emphasizes the deduction that the potentials observed as electrotonus of the roots are an index of changes in localized parts of the cord (presumably where their fibres end) and that the roots act as specific leads to these regions and are little affected by changes in other regions close to them.

Summation of dorsal root potentials

The magnitude of potential change alters when the strength of the stimulus is changed. Fig. 4 shows that as this is increased the potential increases in height to a maximum but does not show any change of form. The size of the maximal potential observed in any rootlet varies when the volley evoking it enters by different rootlets; if a large rootlet be stimulated the observed potential is greater than that following stimulation

of a small rootlet, but the increase is not in proportion to the size and the potential tends to a maximum when rootlets more than about 1 mm. in diameter are stimulated.

Throughout the work described in this paper, unless the contrary is stated, the electrical stimulation was adjusted to be just supramaximal for the production of the central slow negativity.

If two maximal volleys are sent in simultaneously by different large rootlets the potential produced by both together is little or no greater than the larger of the potentials, if unequal, produced by the two volleys

Fig. 4. Dorsal root potentials: single break shock to an adjacent root, increase of strength of stimulus up to that just producing maximal dorsal root potential in C and F. A, B, C, cat, electrodes on root, cut peripherally, 1·5 and 14 mm. from cord. D, E, F, frog, 15° C., ditto 1·9 and 12 mm. from cord. (Time marker shows 0·1 and 0·02 sec. intervals.)

separately, and is thus much less in height and area than their sum. But when both volleys are submaximal there is appreciable summation, but the potential from both volleys together is still much less than the sum of the potentials which they produce in isolation. The dorsal root potentials thus exhibit spatial summation and occlusion, and increase in size towards a maximum when the number of fibres stimulated is increased, whether this be effected by an increase in a submaximal stimulus, or by changing the stimulus from a small rootlet to a large one, or by stimulating several rootlets simultaneously.

Temporal summation

When two successive maximal volleys are sent into the cord by the same fibres partial summation occurs, but the second produces a much smaller potential time area than when it occurs alone, the extent of this

reduction depending on how closely it follows its predecessor (Fig. 5). The earlier it occurs the smaller is its effect. Thus it is not possible to determine sharply a refractory period following the first volley after which summation appears; detectable summation of the effect begins to occur for volleys separated by 3 msec. in cats and 6 msec. in frogs. However, the mode of summation is much clearer from consideration of the results of repetitive stimulation recorded with direct coupled amplification (see Figs. 6, 7). If the second volley arrives before the negativity produced by the first has died away, a maintained negativity results.

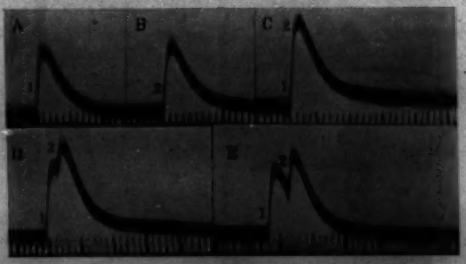

Fig. 5. Potential evoked in a dorsal root when two successive volleys enter the cord by an adjacent root. Frog, 15° C. Recording electrodes on cut root 1·9 and 12 mm. from cord. Coil primaries broken by Lucas pendulum. A, 1st stimulus alone. B, 2nd stimulus alone. C, 1st followed by 2nd at 0·02 sec. D, ditto, 0·038 sec. E, ditto, 0·082 sec. (Time marker shows 0·1 and 0·02 sec. intervals.)

At high rates of stimulation this produces a summated negativity which lasts as long as the stimulation, even up to duration of minutes (see Figs. 6, 7 C). Prolonged stimulation in cats is often followed by a wavering potential of variable pattern (Fig. 7 C, D, E), and in frogs the negative potential takes many seconds to subside (see Fig. 6 E). Summation of repeated volleys entering the cord by contralateral roots occurs in exactly the same way. Similarly, maintained negativity results from prolonged sensory stimulation. This is illustrated in Fig. 6 F.

A steady stream of incoming impulses is thus able to produce a steady negativity in fibres terminating in the cord. In the cat a change of position of a limb will produce a shift in the root negativity, which will remain and fall very slowly until the limb is returned to its original position. In the frog a change occurs when the limb is moved but gradually dies away. This may well be due to the fall in sensory input

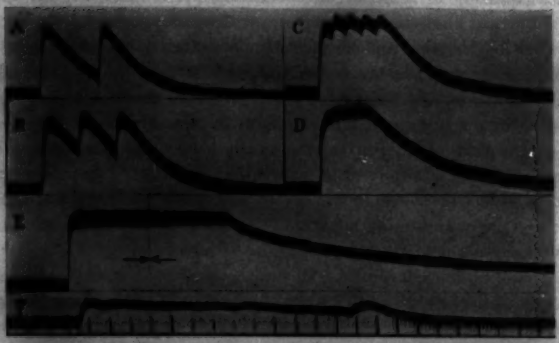

Fig. 6. Temporal summation of dorsal root potentials. Frog, 12° C. Electrodes on cut root VII, 1 and 12 mm. from cord. Stimulation by neon lamp stimulator to adjacent dorsal root. A, at 3·3 per sec. B, at 5 per sec. C, at 14 per sec. D, at 28 per sec. E, at 80 per sec. for 30 sec., 29·5 sec. of record omitted between arrows. F, sensory stimulation by 10 g. weight placed on ipselateral foot for 1·4 sec. (Time marker shows 0·1 and 0·02 sec. intervals.)

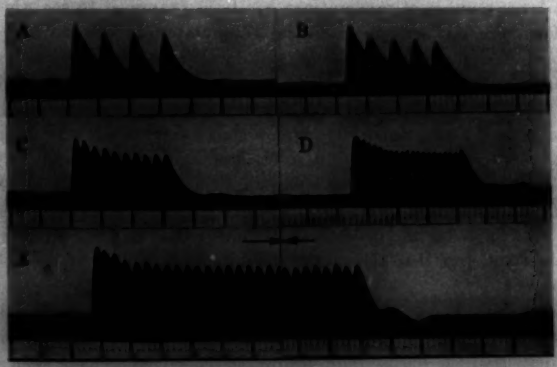

Fig. 7. Temporal summation of dorsal root potentials. Cat. Electrodes on cut rootlet 1·4 and 12 mm. from cord. Stimulation by electrodes on adjacent root. A, at 10·7 per sec. B, at 14·3 per sec. C, at 33 per sec. D, at 49 per sec. E, at 33 per sec. for 15 sec., 14 sec. omitted between arrows. (Time marker shows 0·1 and 0·02 sec. intervals.)

from adaptation of sense organs occurring much more rapidly in the frog than cat. The sensory impulses are thus reappearing as integrated states visible in the root negativity. We have sometimes used one amplifying stage condenser coupled to eliminate such long term effects from our records, and sensory stimulation has been such as added impulses to the sensory input (pinching foot, traction of a particular muscle, acid on the skin, etc.). But as long as the cord is in connexion with its sensory fibres it is receiving a vast input of sensory impulses which presumably act in the same way as do any further additions of sensory input and are maintaining negativity, so the condition for zero sensory inflow may be much less negative than the normal state. We have tried to determine whether cutting down the sensory input permanently reduces the negativity of the roots, by sectioning the sciatic nerve in curarized animals. After the potential evoked by the volley caused by sectioning the nerve, the level usually falls slowly below the original level, but owing to amplifier instabilities we have not been able to make a reliable estimate of the magnitude of this effect. It thus appears that trains of impulses determine the level of negativity of some regions of the cord and that as long as the sensory input is maintained these persist.

Potentials of opposite sign

A large number of preparations has been examined to see whether any form or site of sensory or electrical stimulation produces a potential of opposite sign or reduces the normal dorsal root negativity following a sensory volley. No stimulus of any kind which added impulses to the total input has been found to have any such effect but it always produces or increases the root negativity. Fig. 8 shows summation and occlusion of the negativity produced by ipselateral and contralateral volleys. This occurs no matter how the volleys are timed relative to each other, but if the volleys are coincident the summed potential is little greater than that following the ipselateral stimulus alone, and it is always less in height and area than the sum of the potentials evoked by the volleys in isolation.

As shown above, if a muscle be stretched a persistent negativity in the roots results and when the muscle is released this negativity subsides. Had the release only been observed it might have been concluded that it caused a positive polarization. This probably accounts for small positive deflexions which sometimes follow limb movement, indicating that the movement has resulted in a reduction in the sum total of sensory input, to that part of the cord where the rootlet under

observation terminates. In cats a small deflexion of opposite sign and variable form sometimes follows prolonged stimulation (Fig. 7).

Our results are in fact a confirmation of the observations of Gotch & Horsley [1891] made nearly 50 years ago with a mirror galvanometer. These authors found in the monkey and cat that the central of two electrodes on a dorsal root became negative during any form of stimulation but never positive.

Fig. 8. Dorsal root potentials: concurrence of ipsi- and contralateral volleys. Frog, 13° C. Electrodes on cut dorsal root 0·8 and 12 mm. from cord. Stimulation of adjacent ipsi- and contralateral roots *I* and *C* in records. A, *I* followed by *C* at 0·021 sec. B, ditto, 0·102 sec. C, ditto, 0·293 sec. D, *C* followed by *I* at 0·173 sec. (Time marker shows 0·1 and 0·02 sec. intervals.)

Depressants

The dorsal root potentials (e.g. those evoked by a tendon tap or single volley evoked electrically) are abolished by very deep anæsthesia or by asphyxia, and in the frog by nicotine as well. When evoked by repetitive stimulation, three to five per second, the first negative wave after a period of quiescence is usually larger than succeeding waves, thereafter there is a slight reduction to a height which remains steady, some 70 p.c. that of the first; this reduction with repetition becomes much more marked in preparations which are in poor reflex condition. It appears to indicate a fatigue process.

Part II. Electrotonus in ventral roots

As was reported by Umrath & Umrath [1934], large slow potentials may be led off from the ventral roots of the frog's spinal cord, and these potentials correlate with reflex contraction of muscles. We have repeated, confirmed and extended these experiments on the frog and made similar studies on the cat, but we find that in the ventral roots as in the dorsal roots, the cord potentials are conducted

along the root by electrotonus, and not, as the Umrath's suggested, by simple electrical spread.

Prolonged stimulation via sensory nerve endings

In Fig. 9 are shown the slow negative potential and the impulse discharge recorded from a ventral root in a frog when the foot is squeezed; in A the pressure is increased gradually and suddenly released; in B it is suddenly applied and gradually released. The impulse discharge is always preceded by a slow negative change, and only after this has developed to a certain height do the motor neurones start to discharge. In Fig. 9 C

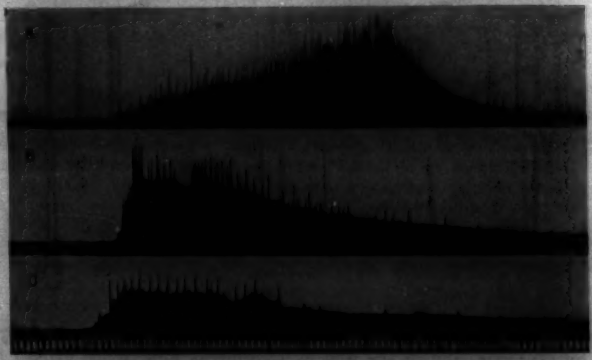

Fig. 9. Electrical changes in ventral roots. Upward deflexion = negativity of proximal electrode. Spinal frog, 15° C., A and B electrodes on cut root 3 and 9 mm. approx. from entry point of root to cord. Stimulus pinching of ipsolateral toes. A, pressure increased gradually and suddenly removed. B, pressure suddenly applied and gradually removed. C, frog 13° C., electrodes 3·4 and 12 mm. from cord. Nerve subdivided between electrodes and cord to reveal response of a single unit. Stimulus 50 g. weight placed and left on foot. (Time marker shows 0·1 and 0·02 sec. intervals.)

is shown the impulse discharge after the root has been subdivided, so that the response of a single motor neurone reaches the recording electrodes. The leads are close (proximal about 3 mm.) to the cord, and the impulse and coincident electrotonic potentials are recorded. This latter potential is not necessarily originating in the same fibres as are the impulses, for, as is well known, electrotonus spreads from fibre to fibre.

Similar potentials recorded from the cat are shown in Fig. 10 during a flexion reflex; Fig. 10 A was recorded from a small rootlet about half

a millimetre in diameter; in B the rootlet has been subdivided and the response of two motor neurones was recorded showing recruitment of the second when the slow depolarization reaches a certain height. This is a common feature of all our observations. If the sensory stimulus is gradually increased, the potential level rises gradually, and motor neurones recruit as it rises, they fall out again in the same order as the level falls, though they often do so a little before the potential has fallen to the point at which they commence to discharge. This can be seen in the records illustrated.

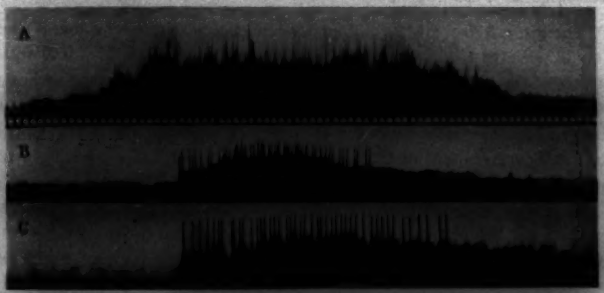

Fig. 10. Electrical changes in ventral rootlets (L_6) of a decerebrate cat. Upward deflexion =negativity of proximal electrode. A, small severed rootlet, electrodes 3 and 14 mm. approx. from cord. Stimulus squeezing of toes. B, ditto (electrodes at 3·5 and 13 mm.) after subdivision of root between electrodes and cord. C, ditto after further subdivision. (Time marker shows 0·1 and 0·02 sec. intervals.)

To obtain Fig. 10 C the nerve was further subdivided and the response in a single motor fibre recorded. In both frog and cat there is a close correlation between the magnitude of the negative potential and the frequency of response of the motor units, though at any level of potential the frequency is usually lower if the level is steady or falling than if it is rising. After prolonged stimulation the slow potential may take some seconds to die away completely, particularly in decerebrate as opposed to spinal cats.

The slow potentials spread along the roots by electrotonus and the relative heights, of impulse and electrotonic potentials can be adjusted by moving the electrodes towards or away from the cord. The impulse potentials remain the same and the slow potentials increase in magnitude as the proximal electrode approaches the cord.

In practice the sensitivity of the amplifier is adjusted so that the impulses are clearly recorded and the electrodes moved until the electrotonic potential is of such a size that it also appears clearly in the record of this sensitivity. In a few experiments we have used two recording systems, one coupled by small condensers so that it records only impulse potentials, the other direct coupled; the size of impulse and electrotonic potentials can thus be adjusted independently without moving the electrodes, but we have not found the additional clarity obtained by a double record sufficient to justify the added complications of dual recording.

As a small electrode shift causes a considerable change in the size of electrotonic potential (rather more than double per 1·5 mm.), and since it is difficult to measure the electrode distances from the cord with great accuracy (e.g. fluid on nerve, stretch of nerve), the electrode distances given throughout are approximate and the exact absolute magnitude of the deflexions is not of significance.

The largest electrotonic potentials in cats are seen in the sacral roots. This is perhaps to be expected, for here the grey matter is close to the surface of the cord, so the electrodes on the root are closer to the presumed site of origin of the potentials here than elsewhere. The greatest potentials observed from ventral roots are about 4 mV. in frogs and 2 mV. in cats, but such large potentials are seen only with electrical stimulation (see below).

Prolonged sensory stimulation (e.g. squeezing the foot) apparently results in a prolonged depolarization of the motor neurones with the coincident impulse discharge which in decerebrate cats often lasts as long as the stimulus is applied. With leads from which the slow potential is seen, discharge from motor neurones evoked by any form of sensory stimulation never occurs unaccompanied by the slow potential, but conversely a small slow potential unaccompanied by impulses is easily evoked by weak sensory stimulation of a small area.

Brief sensory stimuli

In Fig. 11 A–D are shown the potentials resulting from brief sensory stimulation in the cat. A tap over the patellar tendon evokes a discharge of impulses preceded and accompanied by a negative potential. Most of the response usually occurs on the rise of this potential, which occurs very rapidly, it starts to fall abruptly, but often takes 100–200 msec. to disappear completely. The potential does not fall steadily, often wavering as in Fig. 11 B. In Fig. 11 E is shown the response in a frog to a tap on the foot. In no preparation of cat or frog stimulated thus have we seen an impulse discharge not preceded and accompanied by depolarization.

We have not specially investigated changes in the mode of response in decerebrate animals after the cord is sectioned in the mid-dorsal

region, but we have often observed that the fall of potential (e.g. Fig. 12 A) occurs very abruptly if the spinal cord has been sectioned. And in spinal animals the changes resulting from prolonged stimulation are maintained for only one or two seconds. The electrotonic potentials are always of the same sign, the proximal electrode becomes negative to the distal during activity. The slow deflexion is thus in the same direction as the monophasic impulse potentials recorded simultaneously.

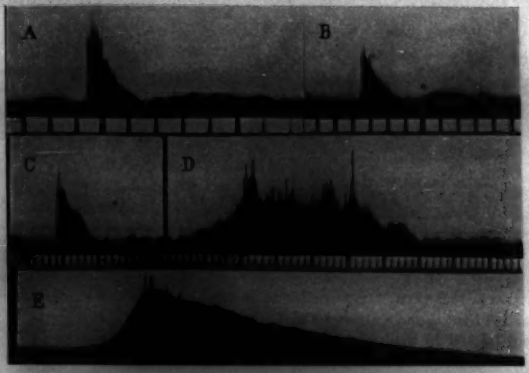

Fig. 11. Ventral root potentials in cats to brief sensory stimuli. A, electrodes 2 and 12 mm. from cord, on ventral rootlet of L_6 stimulus tap on patellar tendon. Cord cut mid-dorsally. B, ditto. C, decerebrate cat. Cord not cut. Ditto. D, ditto. Stimulus tap on base of tail. E, spinal frog. Electrodes 2 and 10 mm. from cord. Stimulus tap on foot. (Time marker shows 0·1 and 0·02 sec. intervals.)

No form of sensory stimulation has been found to produce deflexions of the opposite sign, for though, as will be shown later, a reduction of the impulse discharge and depolarization produced by one stimulus may often be brought about by a second stimulus in a different region, this second stimulus when applied alone does not produce an electrotonic potential of opposite, but of the same sign (central depolarization).

Electrical stimulation in the frog

In the frog the normal responses to electrical stimulation are similar to those following sensory stimulation. A single afferent volley evokes a slow depolarization which precedes and accompanies a small impulse discharge. This response is fairly constant over long periods (Fig. 12 A). Often a single shock produces a slow depolarization but no impulse

discharge (Fig. 12 C), but the time course of the potential shows considerable differences when different roots or peripheral nerves are stimulated, and occasionally alters with changes in the strength of stimulation. No simple analysis of these differences has yet been found possible, but all records (with exceptions discussed in a later section) lie between the extremes of Fig. 12 A, C. The latency in C is short (4σ), the rise steep,

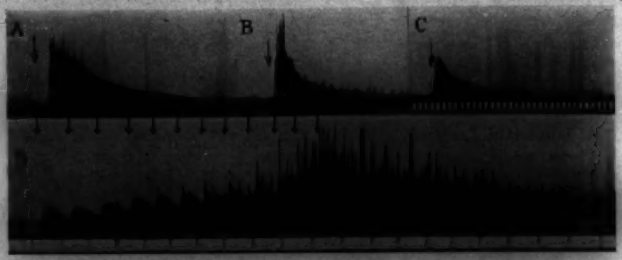

Fig. 12. Electrical changes in anterior roots of the frog in response to shock excitation. Ventral roots cut. A, 14° C., electrodes 1·5 and 11 mm. Single shock to ipsilateral sciatic at arrow. B, 16° C., electrodes 2 and 11 mm. Single shock to ipsilateral dorsal root. Cord cut through about 2·5 mm. above and below exit of root. C, 16° C., electrodes 1·3 and 10 mm. Single shock to ipsilateral peroneal nerve. D, 15° C., electrodes 2·5 and 11 mm. Repeated shocks to ipsilateral peroneal nerve marked by arrows. (Time marker $\frac{1}{25}$ sec. for A–C at foot of C. $\frac{1}{10}$ sec. on D.)

and the maximum occurs after 24σ, whereas in A the latency is long (22σ) and the time to maximum 90σ. The changes observed are still present after the cord has been sectioned 5 mm. above and below the roots under examination, though the impulse discharge may be thereby increased (see Fig. 12 B).

The segment of cord thus isolated may then be lifted from the frog, and will continue to function thus for several hours if irrigated continually with glucose-gum-Ringer's solution [Kato's formula, 1934] from a flask under a pressure of half an atmosphere of pure oxygen. This forms a very convenient preparation for certain experiments. If the roots are left in continuity with the frog, reflex movements can be evoked by pinching the frog's leg so that the isolated segment need not be regarded as in completely abnormal condition, though clearly the experimental possibilities of the preparation are limited.

With repetitive stimulation temporal summation of the negative potential occurs as is shown in Fig. 12 D; each afferent volley evokes a slow potential change, and summation occurs between them. When summation has reached a certain height discharge by the motor neurones commences and increases with the rise of the potential; the changed potential and impulse discharge outlast the stimulation, and the fall of

the former to its original level may take many seconds (see Fig. 13 D). The degree of synchrony of the impulse discharge varies greatly in different preparations and with stimulation of different afferent nerves (cf. Figs. 12 D, 13). Fig. 13 shows how the ventral root potentials summate at different frequencies of stimulation, and how facilitation is related to them; each increase in the potential has many impulses on its rising phase which give the summated spikes seen in Fig. 13 C, D, but each outburst becomes bigger than its predecessor as the total potential change increases and occurs earlier after the stimulus. There does not appear to be any change in the latency of the slow potential itself to

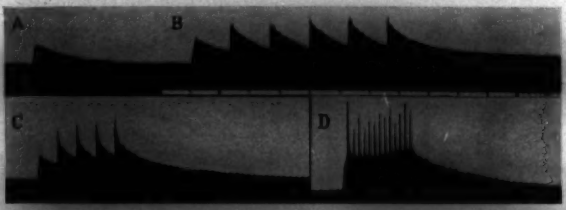

Fig. 13. Frog, 18° C., electrodes on ventral root. 1 and 9 mm. from cord. Stimulation of sciatic nerve. Ventral roots cut. A, single shock. B, 7 per sec. C, 15 per sec. D, 57 per sec. Ripple in base line at 50 per sec. due to artefact from A.C. mains. (Time marker shows 0·1 and 0·02 sec. intervals.)

each successive volley but only of the impulse discharge. But the superadded impulse potentials make each successive rise a little steeper than its predecessor, so that it is difficult to be certain of this. Shortening in latency of the slow potential, if it occurs at all, must be small.

On cessation of stimulation asynchronous after-discharge occurs until the slow potential has declined to about the same level or slightly above that at which discharge of impulses commenced; but there are great differences in the rate of this decline in different preparations (cf. Figs. 12 D, 13 D). The ventral root potentials thus sum, as do those of the dorsal root, but unlike the latter the summated potential in the ventral root may be many times that evoked by a single volley. The frequency in single units and the total magnitude of the impulse discharge is related to the size of this summated potential, but also depends on its rate of change.

Electrical stimulation in cats

If a small sensory rootlet or peripheral nerve twig be stimulated with a single shock the impulse discharge of the motor neurones, as with natural sensory stimulation, is preceded and accompanied by a long-lasting potential change (see Fig. 14). However, the form of the impulse

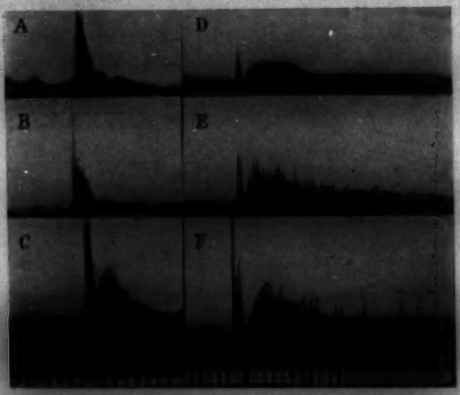

Fig. 14. Ventral root potentials in the cat evoked by single afferent volleys. A–C, decerebrate cat, spinal cord cut mid-dorsally. Ventral roots cut. Electrodes on twigs of L_6, single shocks. A, to small dorsal root twig. B, to ipsilateral popliteal nerve. C, to small dorsal root twig. D–F, decerebrate cat, cord not cut, all from same rootlet. D, single shock weak to ipsilateral popliteal nerve. E, ditto strong. F, shock to large dorsal root bundle. (Time marker shows 0·1 and 0·02 sec. intervals.)

discharge and potential change varies greatly when different posterior rootlets or peripheral twigs are stimulated. The records, while consistent for any given stimulus over considerable periods, vary between the extremes shown in Fig. 14, when the stimulus is applied to different sites and the activity of different rootlets is observed.

This variability of result is perhaps to be expected, for it should be pointed out that the phenomena observed are not, as in myographic reflex observations, associated with the contraction of any particular muscle, for the motor fibres in any motor root are going to a number of different muscles, which will not behave in unison. With small rootlets, however, the majority of the fibres appear to be destined for a particular group of muscles; for this reason we have usually worked with very small rootlets. Stimulation of a dorsal root as opposed to a peripheral nerve presents to the cord impulses arriving by fibres of all functions from a wide peripheral and visceral area. A shock to a collection of fibres which have no peripheral, anatomical or functional association sets up the most completely

abnormal impulse pattern that can be presented to the nervous system, and for this reason stimulation of peripheral nerves where the fibres at least have an anatomical association is to be preferred.

Most records show evidence of two phases of negativity and impulse discharge, each phase having a greater or less prominence in different preparations. The early phase starts 1–5σ after the arrival of an afferent volley, reaches its maximum at 3–15σ and falls towards the original level; it merges into a second phase of much less amplitude but greater duration, that has its crest some 50–75σ after the afferent volley and often dies away so slowly that its full duration is difficult to estimate sharply, but may certainly be longer than 1 sec. The common feature of all records is the potential change that precedes and accompanies the impulse discharge, and the correlation of the impulse discharge with its magnitude, and rate of change. This correlation is brought out more clearly with repetitive stimulation. All the records can be regarded as made up of the early and late phases in varying proportions. The relative proportions of each phase observed from a given motor rootlet differ if the afferent impulses are set up in different dorsal rootlets or peripheral nerve twigs. Thus these differences result from the pathways the afferent impulses have traversed, and are not rigid properties of the motor neurones in any particular root under observation. The later phase of activity is usually more prominent in decerebrate than in spinal animals. If the stimulus is weakened the pattern of activity does not change fundamentally, but the slow changes and impulse discharge are both reduced (Fig. 14 D, E).

Repetitive stimulation in cats

With repetitive stimulation of an afferent nerve temporal summation of the early negativity occurs, but there is little increase in the total height to which it attains over that of the single volley. The later negativity also summates but reaches a much greater height than the single volley height; the impulse discharge correlates with the total negativity. This is illustrated in Figs. 15, 16; Fig. 15 A–D illustrates summation in a preparation showing a large early phase and small late phase, and in Fig. 15 E, F from a preparation showing a larger late effect. In Fig. 16 are shown the similar changes recorded from a single active fibre preparation, in Fig. 16 A early and delayed negativity follow each afferent volley, summation of the latter occurs, and the impulse discharge rises with it. Fig. 16 B, C are from the same preparation when different afferent nerves are stimulated. In B the early negativity is

absent and the rise of frequency as summation occurs shows clearly. In C the response occurs without summation, the negativity is very prolonged and no early phase occurs. The response opens with a curiously

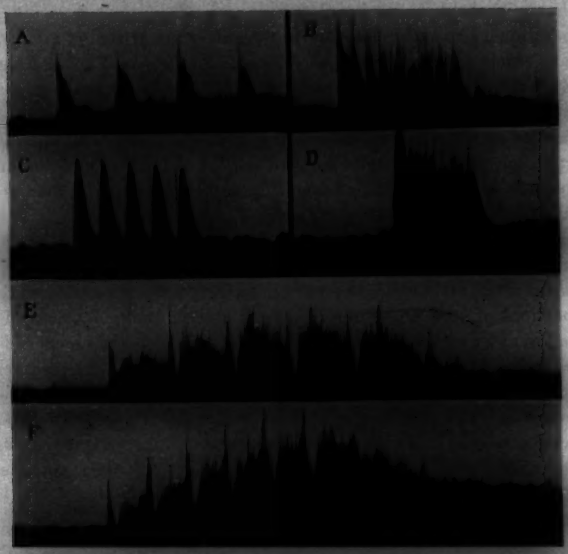

Fig. 15. Ventral root potentials in decerebrate cats. Ventral roots cut. A, B, electrodes on ventral root 1·5 and 9 mm. from cord. Shocks to popliteal nerve. Spinal cord cut mid-dorsally. C, D, decerebrate cat. Shocks to small dorsal rootlet 16 and 61 per sec. Spinal cord not cut. E, F, decerebrate cat. Shocks at 7 and 11 per sec. to ipsilateral popliteal nerve. Spinal cord not cut. (Time marker shows 0·1 and 0·02 per sec. intervals.)

grouped discharge which has been observed in a small number of preparations (cf. the grouped action currents reported by Eccles & Hoff [1932] in the electromyogram of motor units). Generally the slower the rise of the potential the slower its decline, and in preparations such as those of Fig. 16 B, C after repeated stimulation it may take a minute or more to die away completely.

Two other common features are brought out in the records of Figs. 15, 16: first, as the total negativity rises the early phase becomes less

prominent, and secondly, the trough between the early and late phases becomes more prominent as the total negativity rises. With repeated stimulation this trough becomes a definite reduction of the summated negativity towards the original level (cf. the record after the penultimate and last volley in Fig. 15 F; it is clear that the course of the potential is altered by the last volley from the course it would have taken had this volley not occurred).

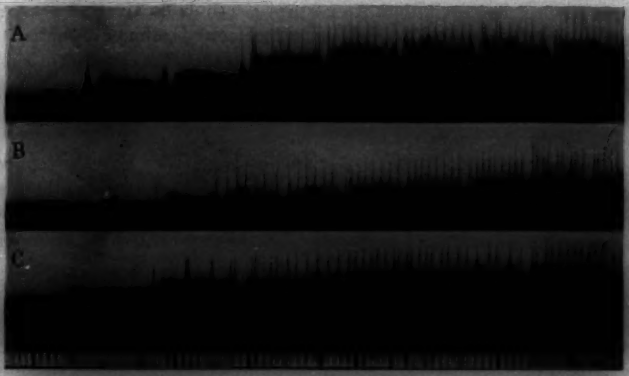

Fig. 16. Decerebrate cat rootlet of L_5 subdivided above electrodes to obtain response of a single unit. Ventral roots cut. A, shocks to ipsilateral popliteal nerve, 4·5 per sec. B, shocks to cutaneous branch of popliteal, 5·5 per sec. C, shocks to a large dorsal rootlet, 2 per sec. Records not comparable as regards amplitude as electrodes were moved between records. Note anomalous opening of discharge in C. (Time marker shows 0·1 and 0·02 sec. intervals.)

"*Detonator response*"

Eccles & Pritchard [1937] have recently repeated some of our observations on the electrotonus of ventral roots in cats and find what they term a "detonator response" occurring with practically no latency. They point out that the latency is much shorter than that of any known reflex; so the phenomenon would appear to be an experimental anomaly and not a component of reflex activity. We have observed such responses only when stimulating large dorsal roots with electrodes close to the cord (see Fig. 14 F). The size of the response varied with the stimulus, but the response of very short latency did not occur when the electrodes were 20 mm. from the cord and the precautions described below were

taken to prevent current spread, nor when the electrodes were on a large peripheral nerve (cf. Fig. 14 E, F).

The effect which we have observed thus appears to be due to spread of the stimulating current to central structures. The roots of the cord seem to allow the stimulating current to spread very easily by electrotonus (see also paragraph below on stimulation of ventral roots). Moreover, great care in the placing of electrodes is necessary when stimulating the dorsal roots, for the central structures seem to have a very low threshold to electrical excitation. An earthed electrode on the root between the stimulating electrodes and the spinal cord is almost essential to ensure stimulation does not take place centrally *via* the capacity to earth of the stimulating apparatus and either earthed leads or leakage (e.g. *via* a moist trachea tube), or in the absence of these *via* the capacity to earth of the cat itself.

In frogs we have observed responses having latency less than 2 msec. only when stimulating dorsal roots in preparations that were otherwise inexcitable and gave no response to pinching, etc. It has sometimes appeared in isolated cord preparations if the oxygenated irrigation was stopped.

Spatial summation and inhibition

When sensory stimulation, e.g. pinching a toe, is applied to two points simultaneously an increase in the slow potential and impulse discharge may result if these two points lie in adjacent areas (two toes of the same foot). But if they are applied on opposite sides of the animal the slow potential and impulse discharge produced by the first alone may be reduced by application of the second, but the impulse discharge is always related to the magnitude of the slow potential, and the second stimulus alone produces a slow potential of the same and never of the opposite sign. We have not worked out in detail the effect seen from concurrence of volleys in different ipselateral nerves; the effects are extremely variable from preparation to preparation. This is perhaps to be expected as stimulation of a nerve trunk results in the simultaneous arrival in the spinal cord of impulses producing excitation and inhibition, and the net result in the particular neurones observed in the ventral rootlet selected will depend on the balance resulting from their concurrence. However, the effects of inhibition by contralateral stimulation are consistent and similar in frogs and cats.

In the frog if a single volley which produces a slow potential in a ventral root be preceded by some 10–200 msec. by a contralateral volley, the slow potential produced by the former alone shows considerable re-

duction, but the latter alone does not produce a potential of opposite sign (see Fig. 17). It must, therefore, be reducing the slow potential of the former by preventing its development rather than by producing an opposing potential. If it occurs after the ipselateral volley it produces a slight addition to the slow potential (Fig. 17 E and F).

The effects of inhibitory stimulation are more clearly seen if a prolonged reflex discharge is set up by ipselateral stimulation, either sensory or electrical, and a contralateral nerve or root is found which when stimulated stops the discharge of motor impulses. The effects are

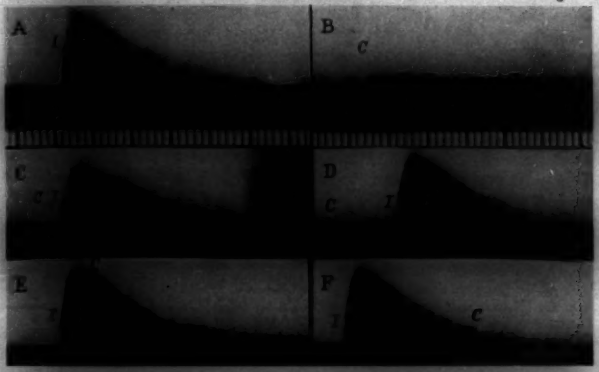

Fig. 17. Ventral root potential in the frog (14° C.). Electrodes on cut ventral root 1 and 10 mm. from cord. Concurrence of volleys set up by single shocks to ipse- and contralateral sciatic nerves. A, shock to ipselateral sciatic nerve. B, shock to contralateral sciatic nerve. C, ipselateral following contralateral after 54 msec. D, ipselateral following contralateral after 167 msec. E, contralateral following ipselateral after 90 msec. F, contralateral following ipselateral after 355 msec. (Time marker shows 0·1 and 0·02 sec. intervals.)

seen most clearly in preparations containing few active fibres, as contralateral stimulation often causes other neurones in the motor rootlet observed to discharge, for the group of fibres observed will supply both flexor and extensor muscles (see p. 294 above). When preparations showing the response of few neurones are recorded the depolarization is reduced by the contralateral stimulation which reduces the impulse discharge. This is shown in the cat in Figs. 18, 19, the potential level and response fall correspondingly. However, the stimulus which produces inhibition, as stated above, when applied alone does not produce a potential change

of opposite but of the same sign, see Figs. 18 D, 19 D. This emphasizes the point made above that it cannot be reducing the potential by an opposing process, but is preventing its development.

In Fig. 19 is shown the inhibition of the response of a single unit by contralateral stimulation, it will be seen that the cessation of discharge is accompanied by a fall in the potential level produced by the excitor stimulus and it builds up again after the inhibitory stimulus is stopped.

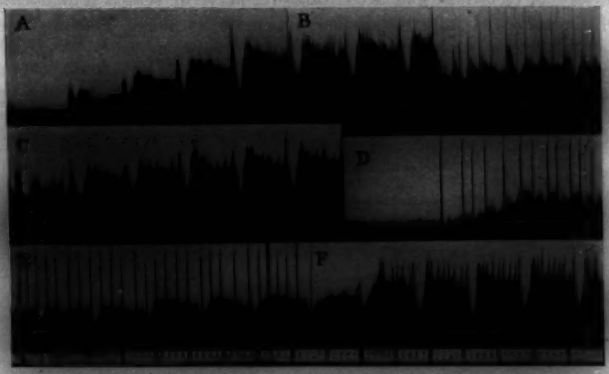

Fig. 18. Concurrence of volleys producing excitation and inhibition in a decerebrate cat. Electrodes on cut ventral rootlet of L_4 approx. 2 and 11 mm. from cord. Repetitive stimulation of ipselateral (6 per sec.) and contralateral (36 per sec.) popliteal nerves. A–C, and D–F are portions of two continuous records. Nerve was irrigated between A–C and D–F, so electrode positions may not be identical. About 1 sec. of record omitted between A and B, B and C, D and E, E and F. A, start of excitatory stimulus. B, addition of inhibitory stimulus. C, cessation of inhibitory stimulus, excitatory continuing alone. D, start of inhibitory stimulus. E, addition of excitatory stimulus. F, cessation of inhibitory stimulus, excitatory stimulus continuing alone. The white line drawn through the whole record to indicate corresponding potential levels. (Time marker shows 0·1 and 0·02 sec. intervals.)

In Fig. 19 E a discharge in a frog's ventral root evoked by a weight placed on the ipselateral foot is inhibited by repetitive stimulation of the contralateral peroneal nerve, this leads to a fall of the potential level towards its resting position and the fall in response accompanies this. Stimulation of contralateral nerves in the frog often produces a very small negativity (Fig. 17 B), but if the stimulus is repeated for a few seconds, some seconds after its cessation a very large potential develops with a large impulse discharge lasting several seconds and this may occur

several times. If during the height of this after-discharge the contralateral nerve is again stimulated the potential falls almost to the resting

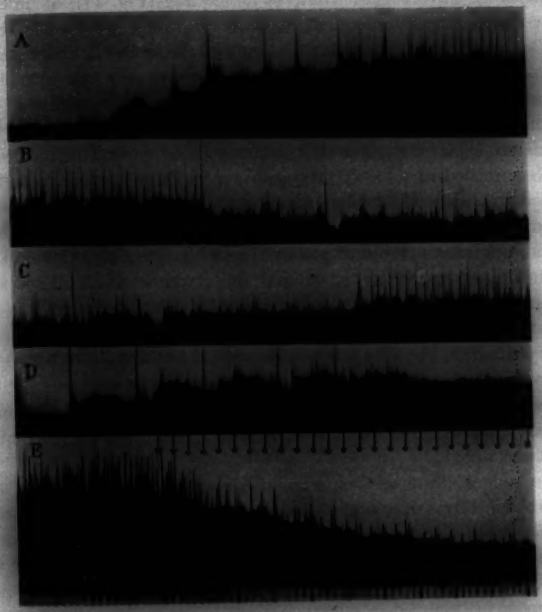

Fig. 19. Concurrence of volleys which produce excitation and inhibition of the discharge of a single motor neurone in the cat. Electrodes on cut ventral rootlet (L_5) 2·7 and 12 mm. approx. from cord nerve subdivided between electrodes and cord. Exciting stimulus shocks 9 per sec. to an ipselateral dorsal rootlet (L_5) inhibiting stimulus 3·2 shocks per sec. to contralateral popliteal nerve. A–C, portion of a continuous record about 0·5 sec. omitted between each. A, start of exciting stimulus. B, addition of stimulus which inhibits response. C, cessation of inhibitory stimulus, excitor stimulus continuing. D, inhibitory stimulus applied alone. White line drawn through record indicates corresponding potential levels. E, frog anterior root electrodes 2 and 14 mm. from cord on cut root. Discharge evoked by a weight placed on the foot inhibited by stimulating contralateral popliteal nerve (23 per sec. indicated by arrows). White line shows resting potential level. (Time marker shows 0·1 and 0·02 sec. intervals.)

level and the impulse discharge is inhibited, and occurs again after cessation of the stimulus.

We have not yet worked out the time course of the effects of concurrence of single excitatory and inhibitory volleys in the cat, but such results as we have suggest that, as in the frog (Fig. 17), an inhibitory volley prevents a subsequent excitatory volley producing the full depolarization which it produces alone. We have never under any conditions seen slow potentials of the opposite sign of a magnitude at all comparable to the negative potentials accompanying excitation. Occasionally a very small positive swing occurs after the negativity, but its magnitude is so small that it is difficult to be certain of its presence. We were misled in some of our early experiments by using condenser-coupled amplifiers which of course can only record potentials that change comparatively rapidly. The use of direct-coupled amplifiers avoids such anomalies, giving a record of potential relative to a resting level. During any stimulus applied to the animal the proximal electrode is always negative to the resting level. Many of the records shown here would indicate fictitious apparent positivities had condenser coupling been employed, e.g. in Fig. 15 F, each volley would be followed by an apparent positivity which would increase with continued stimulation.

Small positivities of slow development may occur and be confused with amplifier drift, but we have not found conclusive evidence of their presence.

We suggest that our experimental results can, at present, all be expressed in terms of a single process, causing central depolarization of the motor fibres terminating in the cord, and that the development of this depolarization can be hindered or prevented by volleys which produce inhibition of the impulse discharge. The mode in which this may be effected is discussed in a later section.

Part III. Excitation of motor neurones

We have shown above that there is a very close association between the slow potentials in the ventral roots and the discharge of the motor neurone. The slow potentials indicate a depolarization of some central portion of those neurones, and the neurones never normally discharge impulses before this depolarization has appeared. Moreover, the rate of rhythmic response of any neurone is related to the magnitude of the depolarization. It thus appears that these potentials are an index of the central excitatory state acting on the neurones for C.E.S. is the hypothetical causal agent of their discharge. It is not necessary to consider here the nature of this central state. Whatever it may be it is clear that it is accompanied by this electrical sign, and excitation does not normally

occur without it. The association of slow potential changes with nerve impulse discharge has been demonstrated in many simpler ganglionic systems [Adrian, 1931; Eccles, 1936]. The present observations show that the excitation of motor neurones of the vertebrate spinal cord is accompanied by electrical changes very similar to those of simpler ganglia.

Eccles [1936] has interpreted his observations on the superior cervical ganglion in terms of two processes *N* and *P*, the former associated with excitation and negativity, the latter with inhibition and positivity. Our observations on the spinal cord agree as regards the association of excitation and negativity, but with electrodes placed on its roots we have not been able to find potentials positive to the resting condition, and while reductions of the negativity produced by stimulation of one nerve often occurs when a second nerve is stimulated (Figs. 17–19), as we have shown above, stimulation of the second alone never produced a positivity, though it certainly can produce inhibition.

It occurred to us that if the depolarization of the motor neurones which we observed as the negativity of the anterior roots were, indeed, the causal agent of the rhythmic discharge of the motor neurones, it might be possible to evoke this discharge by applying an artificial negativity to produce the central depolarization. Experiments showed this to be the case.

Much work has been done, particularly by Lorente de Nó [1935*a*], on the excitation of vertebrate motor neurones by single shocks, but as far as we are aware no attempt has ever been made to stimulate them continuously with steady direct current. Fessard [1936] has found rhythmic movements of insect wings to result from direct current stimulation of central ganglia. We, therefore, explored the possibility of such stimulation of the vertebrate neurones and, as previously reported [Barron & Matthews, 1936*b*; Matthews, 1937], found that continuous response of a frequency depending on the current intensity was produced by direct current excitation. In Fig. 20 is shown the response of a single motor neurone in the frog to various steady currents. Similar rhythmic responses have been observed in single neurones in the cat's spinal cord, the frequency set up varying with the current between 5 and 70 per sec. The arrangement of electrodes, etc. is shown in the accompanying sketch (Fig. 21). The response thus evoked will continue for long periods at a steady frequency, though in a few preparations the frequency has fallen rapidly and ceased after a few seconds. In a preparation with intact dorsal roots it is possible to test the interaction of excitation by afferent

impulses and by direct current. We find that a steady frequency in response to direct current may be increased by simultaneous sensory

Fig. 20. Stimulation of a single motor neurone in the frog (17° C.) by steady current flow. Electrodes as in Fig. 21. A, depolarizing current 5×10^{-7} amp. response 11 per sec. B, depolarizing current 6×10^{-7} amp. response 21 per sec. C, depolarizing current 7×10^{-7} amp. response 32 per sec. D, depolarizing current 10×10^{-7} amp. response 57 per sec. Increase in size in D due to drying of nerve filament. (Taken with one condenser coupled stage in the amplifier 0·1μF. 0·5 megohms.)

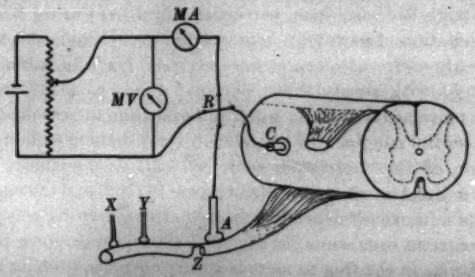

Fig. 21. Diagram to show placing of electrodes to depolarize motor neurones (Fig. 20). A, anode. C, cathode (Zn—$ZnSO_4$—gelatine Ringer—Ringer). *MA*, microammeter. *MV*, millivoltmeter. *R*, reversing switch. *XY*, recording electrodes. *Z*, point at which nerve has been subdivided to cut off all active fibres but one.

stimulation. The current to excite must flow in such a direction that it produces depolarization of the central part of the neurone (cathode on the cord). In the reverse direction a response has occasionally been

obtained, but the current necessary to evoke this response must be then 5 or 10 times as great and the frequency of discharge is high and does not show a smooth progressive increase with the current. However, small currents flowing in this reverse direction have been observed to reduce the frequency of an impulse discharge evoked by sensory stimulation.

This graded responsiveness of the structures of the ventral root to direct current is not shared by the structures of the dorsal root, for they do not respond to similar stimulation, though with currents 20 times greater they often give a rapid discharge for short periods. There is thus some structure peculiar to the motor neurone which very readily responds rhythmically to a direct current. The highest frequency of response which we have observed is about 70 per sec. in the cat, and 60 per sec. in the frog; with further increase of the depolarizing current, the discharge often vanishes abruptly. This would appear to be due to anodal blocking of conduction in the fibres. We have occasionally observed in the frog that rhythmic discharges become intermittent when the current is increased beyond a certain value. This might be due to the presence of collaterals on the fibres acting in the way previously described [Barron & Matthews, 1935], but as its appearance is capricious and the conditions under which it occurs not fully understood, it will not be discussed at present.

Effects of antidromic volleys

In the frog volleys of antidromic impulses set up by stimulation of the sciatic nerve (all dorsal roots cut) produce no detectable slow potential in the ventral roots by which they reach the cord (Fig. 22 A). Nor do they greatly influence the development of slow negativity resulting from a dorsal root volley no matter by what interval they precede or follow the dorsal root volley. While antidromic volleys in the frog do not produce any change observable from two electrodes on an anterior root, they do, as shown by Umrath [1933], produce slow potentials of considerable magnitude detectable from two electrodes on the cord. We have confirmed this and find the same potential also appears in the dorsal roots. This potential resembles that set up by a dorsal root volley in form but is only about one-fifth the amplitude of the latter and has a greater latency, see Fig. 22 C, D. Moreover, unlike the potential resulting from a dorsal root volley it is larger when recorded from two electrodes separated by some 20 mm. on the cord than when it is recorded from a dorsal root in air (Fig. 22 B, C). This potential may perhaps be related to the facilitation of the reflex response described by Klejntjens [1937]

following antidromic faradization in the frog, and to the anatomical fact [Cajal, 1909] that in the frog the ventral horn cells have a branching dendritic system that reaches the lateral and dorso-lateral margins of the spinal cord.

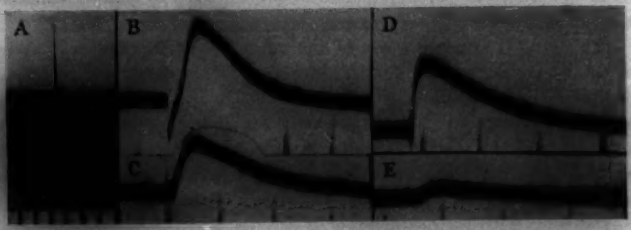

Fig. 22. Potentials evoked in the spinal cord of the frog by antidromic volleys set up by single shocks to the common sciatic nerve, all dorsal roots cut. A, electrodes on a ventral root 1 and 10 mm. approx. from cord. Compound triphasic spike potential of centripetal volley but no slow potential. B, electrodes on side of the cord 20 mm. apart. C, same sensitivity as B. Electrodes on corresponding dorsal root 1 and 8 mm. from cord. D, sensitivity one-fifth that of B and C. Electrodes as C. Potential evoked by single shock to adjacent dorsal root, for comparison of latency with C. E, C repeated at same sensitivity as D, to show relative amplitudes. (Taken with a single stage in the amplifier coupled by 8μF. 0·5 megohm.) (Time marker shows intervals of 0·1 sec. in B–E and 0·02 in A.)

We have confirmed Hughes & Gasser's [1934*a*] finding that no comparable potential occurs in the cat, and we find that antidromic volleys set up by stimulation of the sciatic nerve (dorsal roots cut) produce only a very small change (Fig. 23 A) in the ventral root by which the volleys reach the cord. If, however, the ventral root itself be stimulated peripheral to recording electrodes a very large central potential occurs (Fig. 23 B), which is not observed when the stimulus is applied to the sciatic nerve. The difference might be due to the presence in the root of fibres not present in the sciatic nerve (e.g. visceral efferents), or to spread of the stimulating current by electrotonus to central structures more excitable than the fibres under the stimulating electrodes. This latter alternative appears to us to be the explanation of the difference, as we have been unable to evoke such potentials by stimulation of a root when the electrodes were a long way from the cord (50 mm.) and an earthed electrode was placed between them and the cord.

These potentials evoked by ventral root stimulation have recently been described by Eccles & Pritchard [1937] who suggests they are

"the action current of the motor neurones" and are composed of *N* and *P* waves. We find it difficult to accept such a simple explanation, for while these potentials must clearly be attributed to central structures they result from applying an electrical stimulus to a ventral root rather than from the arrival of antidromic impulses at the cord. Moreover, the positive and negative components cannot have a common site of origin as their observed duration and relative size vary with the position of

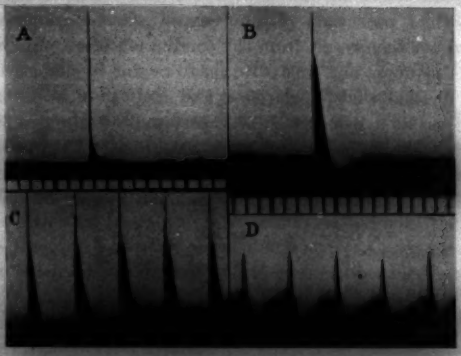

Fig. 23. Potentials evoked in the spinal cord of the cat in response to an antidromic volley and to stimulation of a ventral root. A, electrodes on continuous ventral rootlet 1 and 7 mm. from cord. Response to a single shock to whole sciatic nerve (dorsal roots cut). Diphasic response of centripetal volley not followed by a prolonged change. B, recording electrodes as in A stimulation *via* electrodes on the same ventral rootlet 15 mm. from the cord is followed by a complex slow potential. C, recording electrodes 2 and 8 mm. from cord, repeated stimulation (15 per sec.) of same rootlet by electrodes 15 mm. from cord. D, as in C, but proximal recording electrode moved on to cord some 2 mm. from point of entry of root. Note change of form of complex potential.

the recording electrodes (cp. Fig. 23 C, D). With both leads on a root in air the negative component predominates. As one electrode approaches the cord, the positive component increases and encroaches on the negative component abbreviating it, and further, when one lead is on the cord the negative component becomes very brief and often smaller than the second "positive" component, suggesting that the net potential results from some activity producing a negativity which is conducted in the cord away from the point of entry in the root.

The normal slow depolarization accompanying reflex discharge of the motor neurones in both the frog and cat set up by dorsal root stimulation

is little affected by antidromic impulses set up by stimulation of the sciatic 25 times per sec. (dorsal roots cut). The motor impulses continue to be set up, but the rhythm of the motor neurones is reset by the antidromic activity (as was shown from the electromyogram by Eccles & Hoff [1932] in exactly the same way as the rhythm of a sense organ is reset by an antidromic interruption [Matthews, 1931, 1933]. The resetting of the motor neurone is only clearly seen in preparations containing few active fibres.

The normal potentials of the ventral root accompanying reflex activity cannot then result from the conduction of an impulse over the surface of a neurone, but appear rather to be due to changes external to the motor neurone that depolarize it and cause it to set up a rhythmic discharge, just as a stimulus to a sensory nerve ending appears to set up a rhythmic discharge by depolarizing it. In fact a very close parallel may be drawn between a sense organ and a motor neurone, which is emphasized by a tabulation of their common properties (see Table I).

TABLE I

Motor neurone	Sensory nerve ending
Steady (reflex) excitation yields rhythmic response [Adrian & Bronk, 1928]	Steady excitation yields rhythmic response [Adrian & Zotterman, 1926]
Rate of response rises with increasing excitation [Adrian & Bronk, 1928]	Ditto [Adrian & Zotterman, 1926]
Antidromic impulses reset rhythm with compensatory pause [Eccles & Hoff, 1932]	Ditto [Matthews, 1931, 1933]
Rhythmic response to deformation [Adrian & Bronk, 1928]	Ditto
Natural reflex excitation produces depolarization, rate of rhythmic response increases with depolarization [Barron & Matthews, 1936*b*]	Depolarization precedes response to natural stimulation? (Visual sense cells, Hartline, 1932; Weaver and Bray effect? also suggested by indirect evidence [Matthews, 1931, 1933])
Rhythmic response to steady current excitation. Frequency increases with current [Barron & Matthews, 1936]	Rhythmic response to steady current excitation. Frequency increases with current (Fessard, personal communication)

The only point in the table not having full experimental support is the question of whether depolarization of sense organs always precedes their response. In an attempt to clarify this we examined the nerves to the frog's skin to see if any slow potential were detectable in addition to the usual impulse potentials occurring when the skin is touched. We found that potentials appearing to indicate depolarization of the terminations, and spreading along the nerve by electrotonus did indeed occur. These changes preceded the impulse discharge which was related to their magnitude. We have not, however, been able to devise controls to determine conclusively whether these slow potentials arise in the sensory nerve endings or in other structures in the skin, but our results on the whole support the former alternative. Potentials recorded from visual sense cells by Hartline [1932] suggest a like interpretation as does the cochlea response of the Weaver and Bray effect.

Adrian & Bronk [1928] have suggested that the similar rhythmic behaviour of nerve endings and neurones may well result from the common properties of polarized structures. The membrane theory of nervous action appears to be adequate to account for many central phenomena. Its application will be considered in a later section.

We may perhaps be justified in regarding the secondary neurone at a synapse as a sense organ responding to the level and rate of change of C.E.S. in its immediate vicinity rather than as a structure simply conducting on the impulses arriving by the primary neurones.

Part IV. Interpretation of the dorsal root potentials

It is clear that the dorsal root potentials are an index of the same process as are the "positive intermediary potentials" which Hughes & Gasser [1934*a*] led from the dorsal surface of the cord, and are in fact the same potentials recorded by a more specific lead. These authors stated: "By the designation 'positive wave' no further implication is included than that in leads from the dorsum of the cord the electrode proximal to the root is positive to the distal one." As the potentials are larger when led from a root in air than from the cord surface it seems probable that they reach the surface of the cord *via* the roots. Hughes & Gasser associate these potentials with activity of the internuncial neurones, but we only agree with this association, in the sense that we regard them as the causal agent of this activity, but do not think that there is as yet any conclusive evidence that they arise *in* the internuncial neurones. Below we suggest a hypothesis of their mode of origin which appears to us consistent with the experimental findings of all the authors we have quoted, and which gives a simple interpretation of our own findings. Potentials indicating central positivity are not normally seen when both leads are on a root. The apparent positivities which are recorded with two leads on the cord appear to be due, as was suggested by Hughes & Gasser [1934*a*], to negativity occurring at the more distant electrode and not to a process of opposite sign. We have found with leads on the cord that it is possible to obtain records showing almost any combination of negative and positive deflexions by placing the leads close to the cut stumps of two dorsal roots and sending in volleys by ipselateral and contralateral roots, but from two leads on a single root in air all deflexions indicate central negativity. While positivities of small amplitude may occur and be of great importance in cord activity and give perhaps an index of depressed excitability just as do the after potentials of a peripheral nerve [Erlanger & Gasser, 1937], we have been unable to find

them by our methods. This may, however, merely reflect the inadequacy of the latter.

It is unlikely that the potentials seen in the dorsal root result from the summation of briefer changes occurring asynchronously in the individual neurones, because of the constant time course of the potential under varying conditions and when the stimulus is reduced (Fig. 4). That the potential is due to long lasting changes in each unit and not to a statistical summation of briefer processes has received more definite support from recent observations of Fessard & Matthews (unpublished), who find that a potential of the same time course occurs when a single sensory impulse in one fibre reaches the cord in the frog, the potential appearing to be the electrical sign of a unitary synaptic process since in a single fibre its size and duration are constant.

Whatever the process may be it is clearly capable of temporal summation, so that a steady state of depolarization may be maintained for long periods (Figs. 6, 7, etc.). That this may be a common feature of synaptic action is confirmed by Adrian's [1937] finding that a similar prolonged negativity accompanies the arrival of impulses at the optic ganglion of the water-beetle when the eye is exposed to a steady light.

The streams of impulses entering the cord are thus determining the state of polarization at various points in the central nervous system, and presumably the pattern of this changes with every change affecting the sensory receptors.

Hypothesis of the origin of the dorsal root potentials

Hughes & Gasser [1934 *a*, *b*] attribute cord slow potentials to a mechanism similar to that responsible for after-potentials in nerve; and they attribute their "intermediary positive potentials" to the internuncial neurones, but we suggest that the potentials that we have observed in the dorsal roots arise at the terminations of the dorsal root fibres themselves, by a mechanism similar to that producing the negative after potential in peripheral nerves. This view was discussed and rejected by Gasser & Graham [1933] who argued that as their "intermediary potentials" showed occlusion they could not be arising in the primary neurones. Below we give the reasons why we consider occlusion must occur even in processes at the primary terminations.

If after-potentials are much more marked in small non-medullated fibres than in large fibres [see Bishop, 1936], an impulse arriving by a dorsal root fibre might produce a large long-lasting depolarization of its fine terminations not shared by the medullated portions of the same

fibre. This would account very well for the observed large potentials we have seen in the dorsal root. In fact Bishop [1936] has recently shown that many features of the potential changes in a ganglion system may also occur in a system containing many fine non-medullated fibres (the cœliac nerve) but few if any ganglion cells. Hence it would seem that non-medullated terminations may be the essential structures at a synapse and that the soma of the cells may be of secondary importance [see Gasser & Graham, 1933]. Anatomical considerations, and the many attributes shared by sensory nerve endings and the dendrites of motor cells (Table I) strongly support this view.

In the frog, potential differences as great as 5 mV. have been observed following a single afferent volley. At the fibre terminations some 2 mm. central to the proximal lead, a potential of the order of 10 mV. may well be occurring, suggesting that the fibre terminations are depolarized by at least one-third and perhaps a much larger fraction of the demarcation potential. If, after activity, a depolarization lasting for some quarter second occurs at the termination and not elsewhere in the same structure, current will flow from the resting polarized parts of the fibre to the terminations, causing electrotonus spreading along it. Thus the structure of the dorsal root fibres would lead to the conclusion that the dorsal root potentials arise in this way if we accept the legitimacy of comparison between peripheral and central structures.

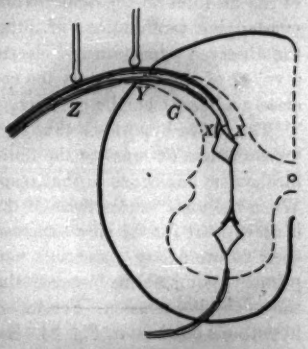

Fig. 24. Diagram to illustrate origin of dorsal root potentials. See text.

The diagram in Fig. 24 shows how we suggest the dorsal root electrotonus arises. In the synaptic region, *X*, the negative after-potential is much greater than that of the larger medullated parts of the same fibre, so that a potential gradient is set up along the fibre which must appear by electrotonus in the extracordal portion *YZ*. The potential indicates a partial depolarization at *X* and a physico-chemical change in this region, since all electrical changes in aqueous media are due to changes in ionic concentration. Moreover, current only flows as ions move, and potential differences exist as relative differences of ionic concentration, unless completely impermeable membranes were present. The root potential

at *YZ* indicates the existence of a potential difference between *Y* and *X*, which is a sign that the ionic differences inside-outside the fibre at *Y* are temporarily not counterbalanced by equal differences outside-inside at *X* and current flows by paths such as *X–G–Y–X*. The slow reversion of the fibre at *X* to its resting condition would give rise to the observed potential which would thus be comparable to a temporary injury potential.

This can also be expressed in the form that permeability reverts to the resting state at *Y* more rapidly than at *X*, hence allowing asymmetrical ionic movements in the system which produce electrical changes. The membrane theory does not define whether the ionic balance conditions the state of the membrane or vice versa, but clearly, the interrelation must be very close. Whatever view is taken, ionic changes must occur outside the fibre at *X* and the inside-outside balance of other fibres in this region must also be affected, so that similar potential differences will be set up in other fibres even though these have not been traversed by impulses, provided, of course, that they terminate close to those along which the impulses have travelled and lie where the external ionic changes are occurring (cf. the effect of changes in ionic composition of the medium on the demarcation potential of an isolated nerve). The overlapping terminations of adjacent rootlets could thus account for the observed appearance of electrotonic potentials in a rootlet whether a volley of impulses enters the cord *via* its own fibres or *via* those of a closely adjacent bundle of fibres.

The above hypothesis thus suggests that the cord potentials arise as ionic changes external to the fibres following impulse activity and by a mechanism analogous to that responsible for after potentials in a nerve. When the ionic implications of this are considered it is clear that there is no support for the view expressed by Gasser & Graham [1933] that their intermediary potentials cannot arise as after-potentials of the primary terminations because simple summation does not occur, and they exhibit occlusion. For indeed occlusion is to be expected on the hypothesis outlined in Fig. 24. Experimentally we find summation does occur if both volleys evoke only small potentials (see p. 283), but stimulation of a moderate sized rootlet (1·5 mm. diam.) produces a maximal effect in roots for some distance up and down the cord, and simultaneous stimulation of other rootlets produces very little increase in the potential observed in any neighbouring rootlet. This may indicate that owing to branching a volley in perhaps 10,000 fibres produces ionic changes over a large area. If now twice as many terminations spread over the same

area are activated by the arrival of an impulse, each termination loses fewer ions, for the ions from the various terminations are escaping into the same space, and so mutually hinder each other. Hence increasing the number of active terminations after a certain point is reached would increase the concentration of ions liberated very little. Spike potentials of nerve do not exhibit occlusion. This may be attributed to the high core resistance of the fibres acting as a limiting factor in the electrical effects external to the fibre, but the distance between the active and inactive parts of the fibre and so the length of core in the circuit is great (wave-length of an impulse in peripheral nerve being some 50 mm.), while under the conditions suggested as responsible for the cord potential

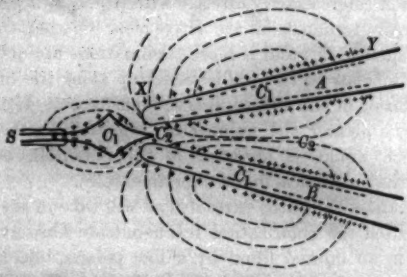

Fig. 25. Diagram to illustrate hypothesis of origin of slow potentials. See text.

the length of nerve between the active and inactive regions may be very short and hence the observed potential may depend much more on the factors external to the fibre.

This point is an important one in any argument involving electrotonic effects in groups of fibres, and hence is set forth below more fully in terms of the membrane hypothesis of nervous activity, in an attempt to show that the effects observed in the dorsal root electrotonus are consistent with, and follow from this hypothesis. Let us consider two semi-permeable membranes (Fig. 25) A and B with a concentration C_1 of some electrolyte inside greater than C_2 the concentration of the same electrolyte outside. (For simplicity only a single pair of ions is considered and not ratios of different ions.) On the membrane theory the ion which can pass the membrane (+) tries to diffuse out owing to $C_1 > C_2$ but is withheld from escaping by the electrostatic attraction of the negative ion restricted by the membrane and a double layer is set up with a resulting potential difference across the membrane. If now an impulse arrives by A, and

the resting state does not revert as rapidly at X as at Y, for a time the escape of ions will continue at X (X may be compared to a temporarily injured fibre), and a potential gradient will exist between Y and X. While X remains permeable, C_1 and C_2 at X will tend to equalize, C_2 will rise. If this occurs in the immediate neighbourhood of another termination B, the local rise in C_2 will depolarize B passively (its depolarization also depending on the difference of C_1 and C_2) and thus the electrical changes seen in A as temporary electrotonus will also appear in B. It is thus clear that electrotonic potentials are to be expected in passive fibres as well as those that have been active if they terminate in proximity. Moreover, their magnitude varies inversely with the difference. C_1–C_2 at X (more exactly directly with (C_1–C_2 at Y)–(C_1–C_2 at X)). However, if many terminations are active close together, C_2 may rise to and become equal to C_1, and if more terminations are active there can be no further increase in the gradients set up along the fibres and as a result the occlusion of any effects observed must occur. Hence we should expect that the greater the number of active fibres the greater the occlusion which would be observed in the potentials accompanying activity, and that is what is found experimentally.

Fig. 25 indicates the paths current-flow will take in the system. The E.M.F. arises from the polarization inside-outside fibre at Y being unbalanced (as in an injured fibre) by a like polarization inside-outside at X. The effects seen in A, the actively depolarized fibre, and B, the passively depolarized fibre, will be very similar, their correspondence depending on the closeness of the terminations; if they were far apart diffusion would cause a lag in the effects in B and a reduction in their magnitude owing to the diffusion concentration gradient. In the cord the close relation of terminations would account for the fact that no such differences in latency are apparent. They may perhaps be revealed by more exact observation, for if they are of the order 1–2 msec. they will have escaped our notice, as the error in measuring latency of a slowly rising process such as the dorsal root potential which does not have a sharp maximum is certainly of this order.

Part V. The membrane hypothesis and excitation of secondary neurones

The secondary neurones lying in the region X (Fig. 25) must also be affected similarly by any ionic changes external to the primary neurones which occur there. The ionic change may thus be regarded as the synaptic transmitter. Electrical transmission at a synapse (unless it

is *via* a metallic conductor containing free electrons) cannot occur except as moving ions constituting an electric current; hence chemical *versus* electrical transmission resolves itself into the question whether whatever crosses the synapse acts in virtue of its charge or its chemical constitution, and whether it moves along a concentration or potential gradient. Practically all chemical reactions in aqueous media occur between ions, so this question becomes one of whether an ion of a *particular substance* or whether any ion carrying the right *charge* is able to activate the secondary neurone. Such a question clearly cannot be settled by electrical observations alone. The transmitter, if an ion, which appears an unavoidable conclusion, has both chemical and electrical attributes. Only the latter are considered here.

In Fig. 25 it is clear that the ionic change at *X* must also depolarize the secondary neurone *S*. We suggest that the electrotonic potentials which we observe in the ventral root preceding the discharge of impulses are due to such depolarization occurring in the neighbourhood of the motor neurones, and that this depolarization causes the motor neurones to respond rhythmically just as it is supposed to do in a sensory nerve ending. We are unable by our methods to observe the impulse activity of the internuncial neurones, but by inference from the motor neurones it may be suggested that the primary depolarization sets up a graded rhythmic response in the internuncial neurones which in turn produces a similar sequence of events at the motor neurone.

Fig. 26 gives a diagrammatic summary of our observations and suggested interpretations. An afferent volley *A* gives rise to depolarization *B* (observed as dorsal root electrotonus). This sets up impulses *C* in an internuncial neurone (suggested by analogy from the ventral root). These impulses produce depolarization *D* (observed as the ventral root electrotonus), which sets up an impulse discharge *E* (observed in the ventral root). Alternatively, if the primary fibre reaches the motor neurone without a relay *F'* and *G'* may occur. We suggest that the two phases of activity which we have observed (Fig. 14) in the ventral root are due respectively to impulses in dorsal root fibres which end directly on the motor neurones and to those which have come *via* internuncial neurones. The diagram illustrates a hypothetical single fibre pathway. Overlapping connexions (not shown) would allow the integration of the activities of different fibres.

It is necessary to consider also the factors responsible for one-way conduction, for in the system suggested it might appear that the depolarization should equally excite impulse activity in all directions, e.g.

B and *S* in Fig. 25 should be equally excited. A possible reason why this should not occur is suggested by the histological structure of the secondary neurone and the primary termination, and by convergence. The termination of the primary afferent neurone alters gradually from a medullated fibre to a fine non-medullated termination, while the motor neurone changes its structure from a non-medullated cell surface to a medullated axon rather abruptly. For the reasons put forward above the gradient of depolarization along the fibre may therefore be less steep in the case of the terminations of the primary afferent neurones and hence would not be as favourable a region for the genesis of impulses as the region of the steeper gradient acting on the motor neurone. However on this subject there is no exact knowledge.

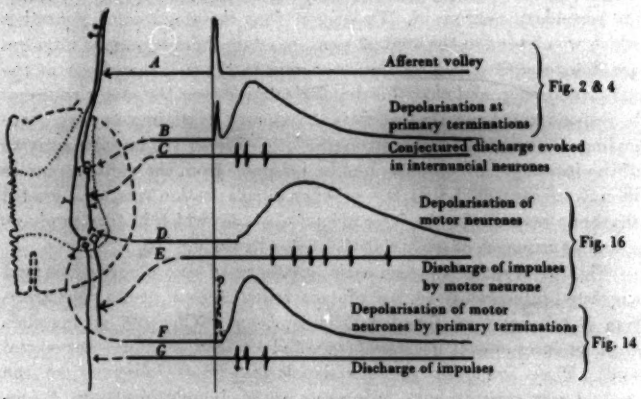

Fig. 26. Diagram summarizing observations and deductions. See text.

If the suggested escape of ions from primary terminations depolarizes secondary terminations and constitutes the synaptic transmitter, it is clear that the very brief escape of ions as an impulse travels over the surface of a neurone is producing a similar change but of a much smaller total magnitude. (There is evidence of this appearing in dorsal root electrotonus in Fig. 4 C, F). But the time-potential areas of a spike potential and the slow potential produced by one impulse in a single fibre in the frog are in ratio of the order 1:300 (Fessard & Matthews, unpublished). Eccles [1937*a*, *b*] has recently suggested that the action current of

a volley reaching the superior cervical ganglion causes a "detonator" response by stimulating the secondary neurones "electrically" (see Fig. 26 F?) and leaves the second neurone facilitated to further volleys. Our own results suggest that in the spinal cord depolarization always precedes the response and that the dorsal and ventral root depolarizations indicate processes responsible for excitation and facilitation. Many observations of reflex summation [Bremer & Moldaver, 1933; Eccles & Sherrington, 1930–1; Bremer & Klejntjens, 1937] agree with this interpretation. The question of the spike activity of reflex arcs has been considered in detail by Hughes & Gasser [1934 *a*, *b*]. Eccles has shown that excitation by spike activity may occur under particular conditions of shock excitation in the superior cervical ganglion, but the delay that occurs in all true reflex arcs shows that the "detonator" response described by Eccles & Pritchard [1937] with a latency of less than millisecond cannot be a normal occurrence in the vertebrate spinal cord. All other impulse discharges which we have observed are preceded by central depolarization. It appears to us that with the arrival at the cord of discharges of impulses the depolarization acting on the secondary neurone builds up outside that neurone and independently of its own impulse activity. The secondary neurone then responds to the depolarization, as a sensory nerve ending appears to do, by rhythmic response. While the depolarization, of which the spike potential is an index, will also contribute to this external depolarization its far smaller magnitude (potential × time) suggests it does not normally have a great stimulating effect. We would suggest that when the secondary neurone is responding rhythmically, after the refractory period following each impulse the excitability of the neurone slowly returns to that level at which response occurs again to the depolarization. An antidromic impulse causes a new refractory period and a slow return of excitability in the neurone to the level at which response again occurs, exactly as in the case of a sensory nerve ending [Matthews, 1931, 1933], and it does not affect or alter the external depolarizing state. This view accounts for our own observations on antidromic stimulation and also agrees with the experimental results of Eccles & Hoff [1932], though the conclusions we draw from them differ from theirs; and agree with those of Lorente de Nó [1935 *b*] who concluded an antidromic volley had no effect on facilitation except by its depression of excitability of motor neurones.

Part VI. Inhibition

The above system makes no mention of one of the most essential features of central processes, inhibition. In a previous paper Barron & Matthews [1935] described a phenomenon of intermittent conduction in the spinal cord apparently conditioned by the state of the grey matter, and suggested that a collateral lying in a region of depolarization might cause blockage of conduction in its parent fibre by the anelectrotonus resulting. More recently we have found further support for this view of the cause of intermittence (in preparation for publication). Intermittence is certainly a mechanism of central inhibition, but that it is the only mechanism could not at present be maintained. It seems probable that after-positivities may be of importance as suggested by Hughes & Gasser [1934*a*, *b*]. But at present many of the reactions of the spinal cord can be accounted for in terms of blockage of conduction by this mechanism [see Hughes & Gasser, 1934*b*], and all the evidence of the present paper agrees with such an explanation. Thus we suggest that inhibition occurs largely in the internuncial neurones *via* their collaterals, but it may occur wherever a fibre gives off a collateral if this collateral ends in grey matter not far from the parent fibre. The negativity at the termination caused by impulses arriving *via* other fibres produces electrotonus spreading back along the fibre. The point at which the collateral leaves the fibre is for physical reasons a region which will be particularly affected by anelectrotonus if other fibres near the T branch are depolarized and negative to their more distant parts (discussion in detail elsewhere [Barron & Matthews, in preparation], see also Rushton [1928] on excitation conditions in a nerve bent at right angles).

That inhibition occurs in this manner is in agreement with our findings on the ventral root electrotonus, that inhibitory stimuli occurring alone do produce depolarization. However, when inhibitory stimuli precede an excitatory stimulus they hinder the development of the depolarization and the impulse discharge produced by the latter. We would suggest that this inhibition is due to a failure of the impulses in the internuncial and direct primary neurones to reach the motor horn cells, a failure brought about by blockage of conduction in these neurones by the slow potentials acting on their collaterals. The fact that we have not observed any mechanism which prevents the production of the dorsal root potentials, whereas such a mechanism is operating on the potentials that may be recorded from the ventral roots, indicates that inhibition

must mainly occur downstream to the structures responsible for the dorsal root potentials.

Our view that the dorsal root potentials are responsible for inhibition is supported by the close resemblance of the time course of inhibition following a single afferent volley [Eccles & Sherrington, 1931, fig. 5] and that of the dorsal root potential in the cat (our Fig. 1 D). Likewise the fall in negativity at the ventral root following repeated volleys (Fig. 15 F, etc.) may be partly due to impulse conduction to the motor neurones *via* internuncial neurones being blocked while the dorsal root potential is at its height.

Thus one and the same process at the fibre terminations, depolarization, is regarded as responsible both for excitation and facilitation of those structures affected by the resulting change in ionic concentrations, and also for inhibition of conduction in more distant structures affected by the current flow external to the depolarized terminations. On this view the pattern of cord activities will to a considerable extent be determined by the relative positions of the structures concerned. In a three neurone arc there will be two opportunities of summation, facilitation, and inhibition, and interaction will be necessarily complex. But we suggest that many properties of the spinal cord can be adequately interpreted in terms of these mechanisms of depolarizations acting on structures that respond rhythmically like sense organs.

So far, therefore, as our observations go they are in general agreement with the known phenomena of reflex activity. Further comparisons of reflex phenomena and the root potentials emphasize their interrelationship, and shows that the above theoretical treatment is in accord with the majority of reflex observations.

Summary

1. Slow potential changes which occur in the dorsal and ventral roots have been recorded in frogs and cats.

2. These potential changes are not due to summed impulse potentials passing the electrodes, but appear to be due to spread by electrotonus of potential changes occurring in the grey matter of the spinal cord.

3. The slow potential changes are set up in any dorsal rootlet when impulses enter the spinal cord by this same rootlet or by neighbouring ipse- or contralateral dorsal roots. In the latter case there is a greater latency.

4. The sign of these potential changes is always the same, negativity of the electrode nearest the cord, no matter what type of stimulation is applied to the peripheral field.

5. The phenomena of spatial summation and occlusion are exhibited by the slow potentials of the dorsal root. They also undergo temporal summation; continued stimulation yields a change of potential lasting as long as the stimulus.

6. No stimulus of any kind, however, appears to hinder or prevent the development of these slow potential changes in the dorsal root, which follow the arrival of impulses from the periphery.

7. Slow potential changes also occur in the ventral root, again spreading by electrotonus. They are always of the same sign; the proximal electrode becomes negative to the distal.

8. These potential changes in the ventral roots precede any discharge of impulses and the frequency of the impulse discharge is related to the level and rise of the potential.

9. Unlike the potentials of the dorsal root, the potentials of the ventral root may be reduced or their development hindered by stimuli which inhibit impulse discharge.

10. However, no peripheral stimulation has ever been observed to produce a potential change in the ventral root of the opposite sign, i.e. proximal electrode positive to the distal.

11. Antidromic volleys *via* the ventral roots produce only a very small potential change in the ventral roots and do not greatly affect the development of, nor reduce the slow potential rise which precedes the impulse discharge of a motor neurone. Such a volley resets the rhythmic discharge of any single motor unit.

12. The behaviour of motor neurones and sense organs is compared, and their many similar properties are attributed to a common mechanism. The motor neurone is regarded as being like a sense organ, responding to the level of C.E.S. in its immediate surroundings, and depolarized by afferent impulses as is a sense organ by its appropriate stimulus.

13. The slow potentials in the dorsal roots are interpreted as having an origin similar to that of the negative after-potential in peripheral nerve fibres.

14. The experimental results of this paper are discussed in relation to the membrane theory of nervous action and the suggestion made that many central processes can be interpreted on this basis.

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THE ACTION OF NARCOTICS ON BRAIN RESPIRATION[1]

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Much work has formerly been done on the effects of narcotics on enzyme activity, with the general result [cf. Clark, 1937] that many enzyme actions are inhibited by sufficiently high concentrations of narcotics. Work done in this laboratory has made it clear that specificity of action of narcotics takes place in the brain, the oxidation of glucose and of lactic and pyruvic acids being affected more than that of other metabolites; and it has also been found that among hypnotics of the same chemical type those with greater hypnotic activity have the greater inhibitive action [Quastel & Wheatley, 1932]. These facts acquired a greater biological significance when it was found that the inhibitory action of certain narcotics was reversible [Quastel & Wheatley, 1934].

To establish that the biological action of narcotics is due to an inhibitory action of narcotics on oxidations, it is necessary to show that narcotics inhibit oxidations at concentrations which bring about narcosis in the intact organism. Preliminary experiments have shown [Jowett & Quastel, 1937*a*] that three narcotics investigated do in fact inhibit measurably the respiration of brain slices at concentrations of the correct order of magnitude.

In the present work a more extensive investigation on similar lines has been made. The effect of narcotics on the respiration of slices of the grey matter of the cerebral cortex has been examined in the presence of glucose, which is the main fuel of the brain. The glucose-oxidizing system of brain is very labile, and the most significant results will be obtained under conditions which damage nerve cells as little as possible. For

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experiments *in vitro* the tissue should therefore be employed in the form of slices.

The technical aspects of the work are of importance, as is shown for instance by the fact that respiration under the present conditions is more sensitive to ethylurethane than it was found to be by Bülow & Holmes [1932], who used minced brain.

Methods

The methods used are essentially those of Jowett & Quastel [1937*a*]. The manometric technique of Warburg is applied to slices (about 0·3–0·4 mm. thick, and of total dry weight 8–15 mg. in each vessel) of the grey matter of the cerebral cortex, shaken in 3 c.c. of a suitable medium. Young rats (80–150 g.) have been mainly used.

The medium is isotonic with 0·16 *M* NaCl, and contains 0·0128 *M* K^+, 0·001 *M* Ca^{++}, 0·0008 *M* Mg^{++}, and 0·02 *M* phosphate of *p*H 7·2, together with NaCl and 0·01 *M* glucose. Solutions of narcotics are made up in 0·16 *M* NaCl, and are added to the medium so as not to alter its salt or glucose concentration. Few of the narcotics are used at concentrations high enough to make the medium essentially hypertonic. Alcohol (in 50 p.c. solution) and chloretone are added to the medium from a side tube of the manometric vessel a few minutes after the vessels are placed in the thermostat at 39°. Other narcotics, when used, are already present in the medium in which the tissue slices are inserted about 10 min. before the vessels are placed in the thermostat. The atmosphere in the vessels is oxygen, and caustic soda (0·2 c.c. of 2 *N* solution) absorbs carbon dioxide. Readings are begun after 12–15 min. shaking at 39°, and are continued over a period of 2 hr. The respiration in presence of a narcotic is always stated with reference to the respiration of a "control" measured simultaneously and taken equal to 100. The respiration of brain under the conditions used, and in the absence of narcotics, is fairly constant over the experimental period of 2 hr.

Results

Six narcotics which have a generally similar action on respiration will first be considered. Alcohol behaves differently, and will be considered afterwards.

The main features of the action of the six narcotics in question are shown in Tables I and II and Figs. 1–7. All the narcotics exert an inhibitory action on respiration, which increases with their concentration.

TABLE I. The effect of luminal on respiration of rat brain. Average effect for 2 hr. period. Probable errors of the mean values are given where data are sufficient

Luminal conc. g./100 c.c.	Percentage effect	Mean	Inhibition calc.
0·01	−16·6, − 5·9, − 7·6, −19·5	−12·4±2·3	8·3
0·02	−28·7, −13·9, − 3·9, −13·9, −10·2	−14·1±2·8	15·3
0·03	−16·2, −32·7, −23·2, −23·2	−23·8±2·3	21·3
0·04	−22·3, −28·7, −27·7, −19·7, −16·9	−23·1±1·5	26·5
0·06	−42, −34	−38	35
0·08	−49, −45, −42, −49, −43, −49	−46±1	42
0·11	−73·5, −72·5	−73	50
0·15	−81·5, −84·5	−83	57

TABLE II. The effect of narcotics on respiration of brain. Average effect for 2 hr. period

Narcotic	Conc. g./100 c.c.	Animal	Percentage effect	Mean	Calc. inhibition
Chloral hydrate	0·015	Rat	− 9, −14	− 6	7
	0·03		−12, − 4, −15	−10	13
	0·06		−33, −20, −25	−26	24
	0·09		−44	−44	32
Ethylurethane	0·25	Rat	−10, − 2½	− 6	7
	0·5		−21, −15, −10	−15	13
	1·0		−28, −23, −18	−23	24
	2·0		−64	−64	38
Avertin*	0·01	Rat	−13	−13	13
	0·02		−19, −20	−20	23
	0·04		−38, −43	−40	38
	0·08		−75	−75	55
Magnesium ion†	0·0048	Rat	−12, − 1	− 6	6
	0·0096		−18, −14, 0	−11	11
	0·024		−28, −26, −34	−29	23
	0·048		−41, −26, −26	−31	37·5
	0·096		−48, −34	−41	55
	0·0178	Rabbit	−14, −25	−19	17
	0·0298		−14, −35	−24	26
	0·0593		−29, −36	−32	41
Chloretone	0·012	Rat	− 7, −18	−12	15
	0·024		−25, −26	−25	26
	0·0333		−35, −28, −34	−32	32
	0·0367		−43, −37	−40	35
	0·007	Guinea-pig	−17, − 6	−12	16
	0·014		−28, −20	−24	27
	0·021		−38, −41	−39	36
	0·0267		−56, −49	−52	41

* Avertin consists of 2 parts by wt. of tribromoethyl alcohol and 1 part of amylene hydrate. The concentration given in the table is that of tribromoethyl alcohol.

† The "control" medium contains 0·0019 g./100 c.c. of magnesium ion. The concentrations given in the table are those added to the control medium.

Tables I and II give the mean inhibitions exerted by the narcotics over a period of 2 hr. at 39°. The data obtained, as long as the

inhibitions do not exceed 35–40 p.c., can be represented by equations of the form

$$I/1-I=Kc,$$

I being the fractional inhibition of respiration, c the concentration of the narcotic, and K a constant dependent on the particular narcotic and varying also perhaps from one animal species to another. The equation can be derived from the assumption that reversible chemical combination occurs between the narcotic and some component of the respiratory system to which respiration is proportional. That the data

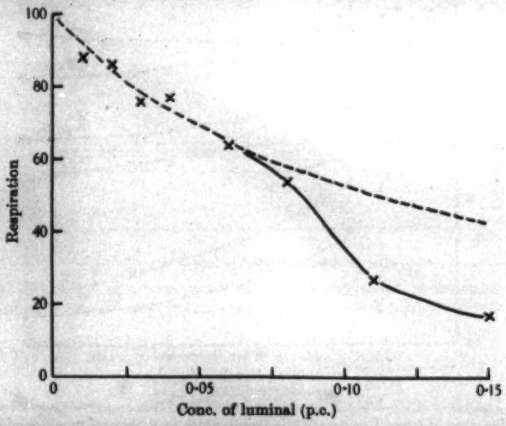

Fig. 1. The effect of luminal on the respiration of rat brain in presence of glucose (mean effect for 2 hr.). The dotted curve is a mass-action curve.

fit the equation does not of course prove the validity of the assumption; the equation however at least serves to represent the data within the experimental error. This may be seen by examining the data in Tables I and II, and also Figs. 1 and 6.[1]

The smaller inhibitions do not vary very greatly with time over a 2 hr. period (Figs. 2, 4, 5, 7). The initially lower values usually observed may be due to the time required for the narcotic to diffuse into the tissue slices. After some time the smaller inhibitions are usually approximately

[1] The data obtained by Jowett & Quastel [1937*a*] with luminal did not appear to fit a mass-action curve. This was due to there being insufficient data for the smaller inhibitions, where alone the relation holds.

steady. The steadiness supports the view that an approximate equilibrium is set up between the narcotic and the respiratory system of the tissue.

When the narcotics are present in concentrations which produce inhibitions exceeding about 40 p.c., definite divergence from the mass-action equation takes place, and in the sense that the observed inhibitions become greater than those calculated. (This is true for the organic narcotics; the inorganic narcotic, magnesium ion, shows if anything a

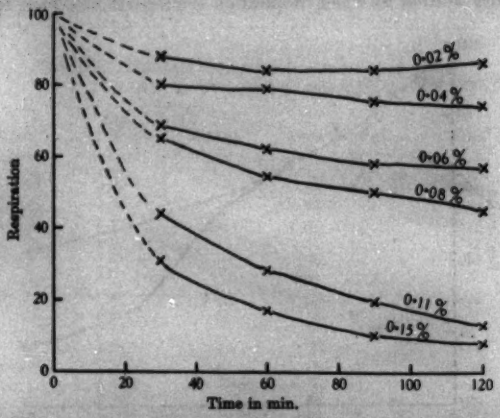

Fig. 2. The effect of luminal on the respiration of rat brain in presence of glucose, as a function of time. The curves are mean values from several experiments.

divergence in the opposite direction.) A clue to the nature of the divergence is found on examining the large inhibitions as a function of time (e.g. Figs. 2, 4, 5), when it is seen that the inhibitions increase considerably as time passes. The nature of the effect is best seen when values of K are calculated as a function of time and of concentration. The results of such a calculation for luminal have been plotted in Fig. 3, which shows that while there is a rough constancy of K for the lower concentrations of luminal, above a concentration of about 0·07 p.c. there is an increase of K progressive with time and concentration. The curves suggest that for a short time after exposure of the tissue to luminal the values of K may be constant even at high concentrations of luminal. The results may be interpreted on the view that a reversible inhibition occurs

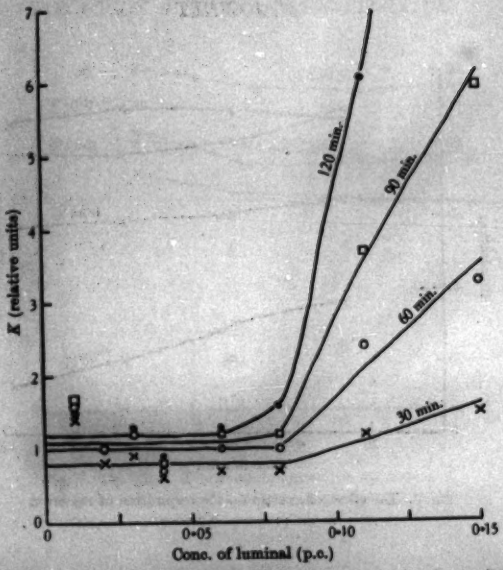

Fig. 3. The mass-action "constants" for the action of luminal on the respiration of rat brain in presence of glucose, as functions of time and luminal concentration.

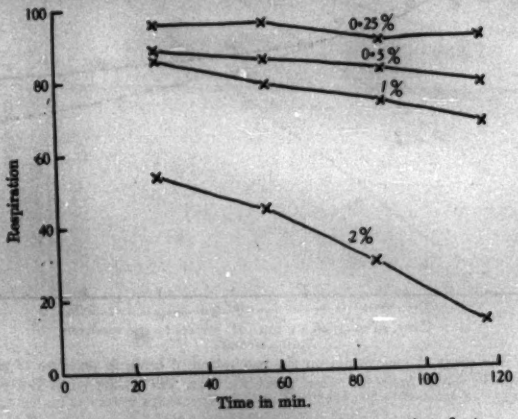

Fig. 4. The effect of ethylurethane on the respiration of rat brain in the presence of glucose.

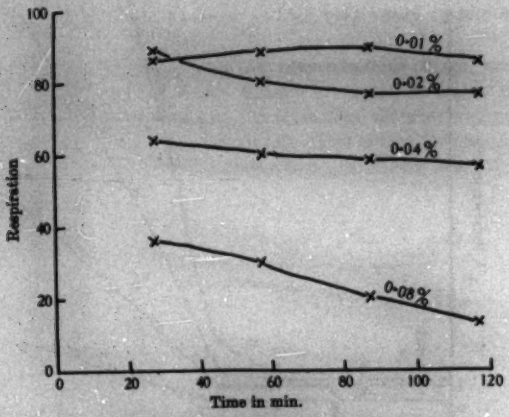

Fig. 5. The effect of avertin on the respiration of rat brain in the presence of glucose.

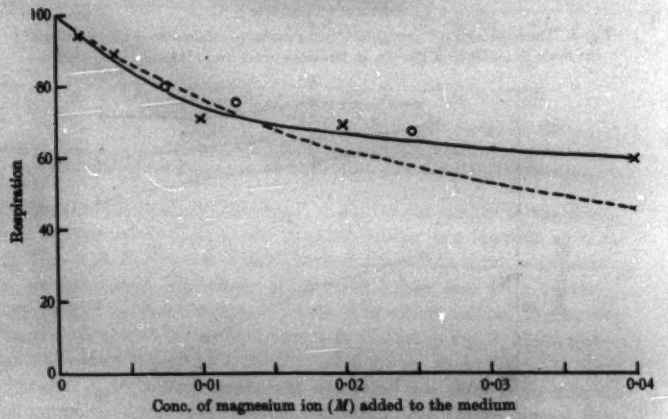

Fig. 6. The effect of magnesium on the respiration of brain in presence of glucose (rat brain ×, rabbit brain ○). Mean effect for 2 hr. The dotted curve is a mass-action curve.

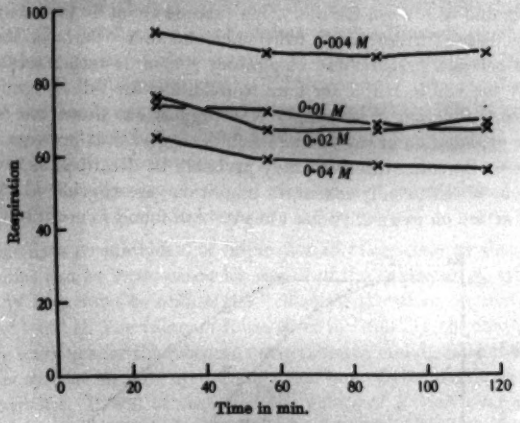

Fig. 7. The effect of magnesium ion on the respiration of rat brain in the presence of glucose.

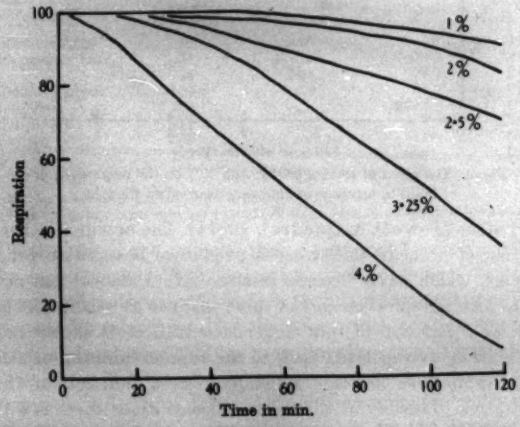

Fig. 8. The effect of ethyl alcohol on the respiration of rat brain in the presence of glucose (at 39°). The curves are smoothed from the means of several experiments.

initially, and that when the inhibition exceeds about 40 p.c. the respiration decreases further in an irreversible manner. Perhaps the equilibrium between narcotic and respiratory system is rather unstable, or possibly the cell is unable for long to maintain the full integrity of its respiratory function if its supply of energy is cut down too far. No definite explanation of the phenomenon is suggested at present.

The six narcotics discussed may probably be described as reversible in action, provided only moderate inhibitions are considered. Another type of action on respiration has already been found to occur in the case

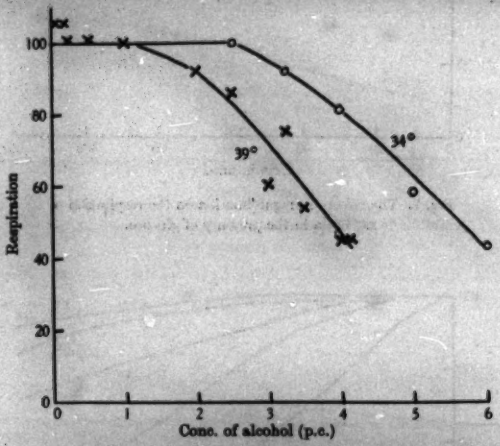

Fig. 9. The effect of alcohol (at 34° and 39°) on the respiration of rat brain in presence of glucose. Mean effect for 2 hr.

of ethyl ether [Jowett & Quastel, 1937*b*]. The action of ether on the respiration of brain in the presence of glucose is to cause progressive inhibitions, which never become steady. Ethyl alcohol has now been found to affect respiration in the same manner as ether. The effect of alcohol, as a function of time and concentration, is shown in Fig. 8. The inhibition, extrapolated back to the time of addition of alcohol, is nil. The inhibitions increase indefinitely as a function of time and concentration. Possibly at the lower concentrations there is a time-lag before the inhibition begins to show itself.

The rate at which the inhibitions develop depends on the temperature. This is shown in Fig. 9, where the mean inhibitions over a 2 hr. period

after addition of alcohol are shown for various concentrations at 39° and 34°. The inhibitions at the lower temperature are much smaller. Ether shows the same phenomenon. In the case of ether the inhibitions have been shown to be irreversible. This is probably true for alcohol also, and so the results are interpreted as meaning that the rate of irreversible inactivation of respiration has a high temperature coefficient.

The effect of narcotics on respiration at concentrations which narcotize the organism

The data on inhibition of respiration of brain slices by the reversible narcotics can be represented by means of the constants K, which have already been used to obtain the "calculated" values for inhibitions in Tables I and II. The values of K are given in Table III, the concentrations of the narcotics for this purpose being taken in mol./l., since the K values in this system of units give a truer measure of the inhibitory power of the narcotic. It will be seen that the values of K vary over a range of nearly two hundred times. The values of K are probably in error on the average by not more than 20 p.c.

TABLE III. Inhibitory activity of narcotics

Narcotic	Mol. wt.	Animal	K	Conc. of narcotic (M) producing 15 p.c. inhibition
Ethylurethane	89	Rat	2·8	0·063
Magnesium ion	24	Rabbit	28·5	0·0062*
,,	24	Rat	30	0·0059*
Chloral hydrate	165	,,	86	0·0021
Luminal	254	,,	230	0·00077
Chloretone	177	,,	253	0·00070
Evipan	260	Guinea-pig	325†	0·00054
Avertin	283	Rat	430	0·00041
Chloretone	177	Guinea-pig	470	0·00038

* Excess above the normal concentration.
† This value has been roughly estimated from data of Jowett & Quastel [1937*a*].

On the other hand no more than a very rough estimate can be made of the concentrations of the narcotics which are in equilibrium with the brain in any definite narcotic state in the intact organism. Estimates have been made by taking data for the doses which produce some degree of narcosis, and assuming that the narcotics are evenly distributed throughout the body. The dose in g./kg. has been assumed to equal the concentration in g./l. Three different errors may make the result inaccurate: (*a*) the depths of narcosis may be variable, (*b*) the narcotic dose may vary from one species to another, (*c*) the assumption of even distribution may be incorrect.

The third source of error is probably the most important. The assumption may give values which are too low, for the narcotic may be distributed only in the water of the body, or may be concentrated at the relevant time in the most vascular tissues. The assumption may on the other hand lead to values which are too high, since absorption may be incomplete at the time considered, or appreciable excretion or destruction of the narcotic may have occurred. What is really required for our purpose is a knowledge (not at present available) of the concentration of the narcotic in the blood—or, more exactly, in the ultra-filtrate of the serum—supplying the brain at the time when the more vascular parts of the brain are in equilibrium with the blood.

The data on narcotic doses taken for the present purpose are given in Table IV. Comment is required with regard to magnesium and evipan.

TABLE IV. Narcotic doses

Narcotic	Dose g./kg.	Administration	Species	Narcosis	Reference
Ethylurethane	2	(Subcutaneous?)	Rabbit	?	Kane & O'Connor [1937]
Magnesium	0·1–0·2	?	?	?	Schmidt & Greenberg [1935]
Chloral hydrate	0·2–0·25	Intraperitoneal	Sheep	Deep, 30–60 min.	Kucera [1933]
Luminal	0·2	?	Monkey	Light surgical	Keller & Fulton [1931]
Chloretone	0·18	Intraperitoneal	Guinea-pig	—	Jowett & Quastel [1937*a*]
Chloretone	0·2	Intraperitoneal	Cat, dog	Satisfactory	Haag & Bond [1927]
Evipan	0·0125	Intravenous	Rabbit	Very light, 10 min.	Jowett & Quastel [1937*a*]
Evipan	0·16–0·45	Intravenous	Rabbit.	Good surgical, 70–195 min.	Maloney & Hertz [1935]
Avertin	0·08–0·10 0·3	Per rectum	Man Rabbit	Basal	Bäyer Bacq & Brown [1937]

In the case of magnesium some information is available regarding the concentration in the blood during narcosis; Schmidt & Greenberg [1935] state that the concentration is 7–21 mg./100 c.c. in the rabbit. Taking the mean as 14 mg. and subtracting the normal blood magnesium, a value of about 0·005 *M* is derived. The same value, it may be noted, would be derived from considering the dosage in this case. This value may not represent very accurately the magnesium ion concentration in the serum, for the available information on the state and distribution of magnesium in blood is suggestive rather than decisive.

As regards evipan, the fact that this drug is given intravenously makes estimation of its narcotizing concentration difficult. Two ways of esti-

mating it, however, agree fairly well. The first assumes it to be given by reckoning a dose producing light anæsthesia for 10 min. to be distributed throughout the blood only. The second assumes that a number of divided doses producing good surgical anæsthesia for some 70 min. are distributed evenly throughout the body.

In Table V are calculated the narcotizing concentrations of the narcotics, and these are compared with the concentrations (calculated from the *K* values) which produce 15 p.c. inhibition of the respiration

TABLE V. Narcotizing concentrations and comparison of narcotizing and inhibitory concentrations

Narcotic	Animal	Estimated narcotic dose g./kg.	Narcotizing conc. (*M*)	15 p.c. inhibitory conc. / narcotizing conc.	Inhibition p.c. due to narcotizing conc.
Ethylurethane	Rat	2	0·022	2·9	6
Magnesium ion	Rabbit	0·15	0·005*	1·2	12
Magnesium ion	Rat	0·15	0·005*	1·2	13
Chloral hydrate	,,	0·22	0·0013	1·6	10
Luminal	,,	0·2	0·00079	1·0	15
Chloretone	,,	0·18	0·0010	0·7	20
Evipan	Guinea-pig	0·16	0·00062	0·9	17
Avertin	Rat	0·3	0·00106	0·4	31
Chloretone	Guinea-pig	0·18	0·0010	0·4	32

* Excess above the normal concentration.

of brain slices in presence of glucose. This 15 p.c. inhibition has been selected as making the two sets of concentrations (in Tables III and V) approximate to equality. The range of the ratios is considerable, but is hardly more than would be expected from the error in estimating the narcotizing concentration.

The result of the comparison is not inconsistent with the view that the seven narcotics considered produce equal inhibitions of some 15 p.c. in the respiration of brain slices when present at concentrations which in the organism would produce an equal fairly deep narcosis. The data do not of course establish such a view, but are sufficient to show that a definite inhibition of respiration is produced by concentrations of narcotics of the order of those producing deep narcosis. The inhibitions, as calculated in the last column of Table V, range from 6 to 32 p.c.

The inhibitory action of alcohol, which has already been shown to be of a different type, does not begin to be appreciable at the narcotizing concentration. Alcohol produces narcosis at a concentration of about 0·5–1 p.c., and reference to Fig. 8 shows that not until the alcohol concentration exceeds 2 p.c. does the inhibition after 2 hr. amount to 20 p.c.

Alcohol is again behaving like ether, for which it has already been found [Jowett & Quastel, 1937*b*] that the narcotizing concentration is a threshold value for inhibitory effects.

Discussion

The view is already held [Winton & Baylis, 1935] that inhibition of oxidations in the brain probably accounts largely for narcotic action. This view is perhaps based on experiments showing diminished oxygen consumption in the brain in the intact organism. It must however be pointed out that no work *in vitro*, prior to the present work and that of Jowett & Quastel [1937*a*], had established that narcotics diminish the respiratory power of tissues at the concentrations at which they act *in vivo*. The use of brain slices has made it possible to show that the respiratory power of the cells themselves is diminished in a direct way by narcotics at the pharmacologically active concentrations.

The action of narcotics on the oxidation of all substrates by brain is not a general one. The oxidations of glucose, fructose, lactate and pyruvate are all about equally sensitive to narcotics, but the oxidations of other substrates, such as succinate and α-glycerophosphate, are much less sensitive.

The mode of action of narcotics on the respiratory systems of cells is as yet unknown. A suggestion as to a possible point of attack has been made [Jowett, 1937], which will be investigated.

The present work strengthens the view that narcotics act biologically by inhibiting cell oxidations. The behaviour of five different chemical types of narcotics is in agreement with the view. On the other hand, alcohol and ether do not inhibit appreciably the oxidation of glucose at their narcotizing concentrations; at higher concentrations they inhibit the oxidation of glucose irreversibly. Alcohol and ether are less polar molecules than the other narcotics investigated, and it may be that this fact is related to their behaviour.

Summary

The effect of narcotics on the respiration of slices of cerebral cortex (mainly of the rat) has been investigated in presence of glucose. The behaviour of narcotics can be divided into two classes.

In the first class, and when the inhibitions do not exceed 40 p.c., the inhibitions of respiration caused by narcotics vary little with time, and are consonant with the view that the narcotic reaches a mass-action equilibrium with some component of the respiratory system, so that the

inhibition is given by a simple mass-action equation. With high concentrations of narcotics (excepting magnesium ion), the large inhibitions observed increase with time, and probably become irreversible.

Narcotics in this class include urethane, magnesium, chloral, luminal, evipan, chloretone and avertin. The inhibitions they produce are measurable (the figures ranging from 6 to 32 p.c.) at concentrations of the same order as those which produce general narcosis in the intact animal. Inhibition of glucose oxidation may therefore be the cause of the narcosis.

In the second class, alcohol and ether (the latter previously investigated) are found. These substances inhibit respiration progressively and probably irreversibly. The anæsthetic concentrations are only threshold values for the production of inhibition of respiration.

I am much indebted to Dr J. H. Quastel for his interest in this work.

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THE BUFFERING OF MUSCLE IN RIGOR: PROTEIN, PHOSPHATE AND CARNOSINE

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In the course of rigor mortis the *p*H of normal mammalian muscle falls from the value in living muscle of 7·0–7·5[1] to a value between 5·5 and 6·0. The extent of the fall is determined by two factors:

(*a*) the algebraic sum of acidic and basic equivalents formed post-mortem; and

(*b*) the buffer curve of muscular tissue.

While it is well known that the breakdown of glycogen to lactic acid is mainly responsible for the change in *p*H, comparatively little is known about the buffering of muscle, the substances responsible for it, and the variations normally met with in the concentrations of these substances.

The buffering in full rigor will differ from that of living muscle in two important respects. First, practically all esterified phosphate will be hydrolysed to orthophosphate; and secondly, any bicarbonate present at death will be destroyed. The buffering system is, therefore, much simpler than that of living muscle and more susceptible of analysis, whilst still affording much information relevant to the conditions present *in vivo*.

It is known that the muscle proteins, myosin in particular [Weber, 1934], are effective buffers in this range, but, as Meyerhof & Lohmann [1926] point out, only about one-half the buffering can be accounted for by the proteins in frog's muscle, and in mammalian muscle in rigor this

[1] The actual value is a matter of dispute. In inert muscle it is probably as high as 7·55 [Voegtlin *et al.* 1934], but Fenn & Maurer [1935] calculate that the *internal* *p*H may be only 6·9.

is reduced to as little as one-third, as can be seen in Fig. 1 which shows the titration curves of a specimen of minced ox muscle and of an ultrafiltrate of the juice expressed from it.

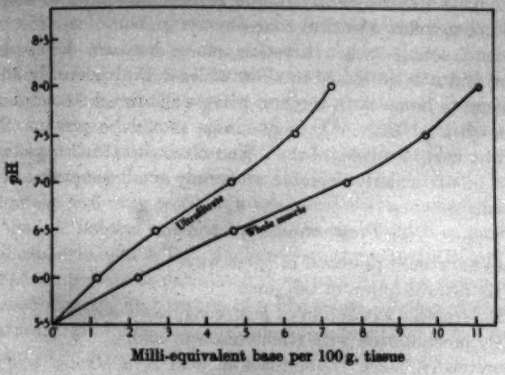

Fig. 1.

Technique

(1) *Titration curves of rigor muscle*

To determine the total buffering power, the muscles are freed from visible fat and connective tissue, finely minced, and a weighed sample of about 20 g. is moistened with a small amount of saline and titrated with 0·1–0·2 *N* HCl or CO_2-free NaOH. *p*H is measured with the glass electrode at room temperature. The most convenient form of electrode for the titration consists of a bulb $\frac{3}{4}$ in. in diameter blown on $\frac{1}{4}$ in. glass electrode tubing. Contact is made internally by means of an Ag-AgCl electrode in 0·1 *N* HCl, and the circuit to the electrometer is completed with a saturated KCl-HgCl half-cell in contact with the brei.

Since equilibrium is attained very slowly in the muscle brei after addition of acid or alkali, it is necessary to ensure that all rapid post-mortem change in the muscle is at an end before beginning the determination. For this purpose, the muscle is kept for 1 hr. at 37° C. in chloroform vapour. In this time the maximum lactic acid concentration is reached and labile phosphorus compounds are converted almost entirely into orthophosphate. In exceptionally well-nourished animals the lactic acid maximum is reached before the muscle glycogen is completely exhausted, and in these cases further formation of acid occurs when

alkali is added. These cases are distinguished by having a *p*H lower than 5·7, and are dealt with by adding alkali to *p*H 7·5–8 and incubating for a further period. The curves obtained by titration of the tissue with acid to *p*H 5·5, and subsequently in the reverse direction with alkali, are then found to follow identical courses.

In constructing such a titration curve, between each addition of alkali or acid it is necessary to allow at least 15, preferably 30 min. for equilibrium to be reached, the brei being well stirred from time to time.

It is essential that no stringy lumps should be present. These are avoided by using a mincer of the "Wedroh masticator" type, which was found to be particularly valuable when only small quantities of material were available.

(2) *Preparation of protein-free extracts*

These have been prepared in three ways:

(*a*) By ultrafiltration of the juice.

(*b*) By aqueous extraction of heat-coagulated muscle.

(*c*) By precipitation with trichloroacetic acid.

Theoretically, since trichloroacetic acid is a strong acid ($pK' < 1$ [Landolt-Börnstein, 1923]) it should have no more effect on the titration of the extract in the range under consideration than the addition of the equivalent amount of neutral salt.[1] This was, in fact, found to be the case. Allowance being made for this effect, no significant differences were observed in the titration curves of the three types of extract, and the last, being the most rapidly prepared, was adopted as a routine method. In preparing the extract care must be taken to restrict dilution. The weighed minced muscle is treated with not more than 50 c.c. of 10 p.c. trichloroacetic acid per 100 g. of tissue, filtered, and a known volume of the filtrate adjusted to *p*H 5 approximately with a few drops of concentrated CO_2-free NaOH, using methyl red as indicator. After cooling, the titration is continued with standard alkali.

In making such an extract, the soluble buffers are diluted in the proportion $x+y/x$, where x is the volume of water per 100 g. of muscle and y the volume of trichloroacetic acid added per 100 g. of muscle. The titration data is corrected by means of this factor in order to make it applicable to the original muscle. It can usually be assumed, with little error, that the water content of lean mammalian muscle is 75 p.c.

[1] The concentration of sodium trichloroacetate present in the filtrate should, by calculation from Green's [1933] formula, lower the pK_2 of phosphoric acid in 0·03 *M* solution by 0·2 approx. This it actually does, as could be shown by titration of phosphate solutions with and without the addition of sodium trichloroacetate.

Following the precedents of van Slyke [1922] and Furusawa & Kerridge [1927], the buffering effect may be expressed as the "buffering power" dB/dpH, where dB represents the mg.-equivalents of base required per 100 g. of muscle to produce an increment, dpH, in pH. The latter authors found in minced cat's muscle a sharp maximum in dB/dpH at pH 6·5. In most species examined this maximum lies between 6·5 and 7, and is largely due to constituents which appear in the extract (the washed, heat-coagulated protein, suspended in water, gives almost a linear titration curve between pH 5 and 8). The position of this marked maximum suggests that the buffering of the extract is largely, or entirely, due to the orthophosphate present in it. It has been shown (cf. van Slyke) that the buffering power of a weak acid is at a maximum at a pH equal to the pK' of the acid, and at the ionic strength of muscle the pK_2' of phosphoric acid is 6·8. If, however, the actual dB/dpH of the orthophosphate present is calculated from van Slyke's data [1922] or is determined directly by titration of a phosphate solution of equivalent concentration, it is found that a considerable proportion of the buffering still remains to be accounted for. An example of this is given in Table I,

TABLE I. Buffering power of protein-free extract of muscle and of the phosphate therein

Range of pH	dB/dpH of		
	Whole extract	Phosphate	Unidentified
5·5–6	1·9	1·4	0·5
6–6·5	3·3	2·0	1·3
6·5–7	4·4	2·2	2·2
7–7·5	3·6	1·4	2·2
7·5–8	2·2	0·6	1·6

in which the dB/dpH of the protein and non-protein fractions of a sample of ox muscle, and of the orthophosphate found therein, are recorded.

A small fraction of the buffering on the more acid side is attributable to lactic acid, which, having $pK'=3·9$, has a feeble buffer action at pH 6, rapidly diminishing at higher pH values. To satisfy the remaining buffering the substance or substances responsible should also, like phosphoric acid, have a pK' between 6·5 and 7·5 and should be present in the particular instance quoted at a concentration of about 0·04 M. The only known constituent which might conceivably satisfy these requirements is carnosine, which is reported to occur to the extent of about 0·5 p.c. or 0·02 M in the muscles of some species, but no reference to the pK' of this substance (which is a dipeptide of β-alanine and histidine) is to be found in the literature. A sample of the base obtained from

the National Institute of Medical Research was found to have a maximum buffering action at pH 6·9, and therefore fulfils in every particular the requirements for the unidentified buffer substance. In the muscles of other species, particularly of birds, carnosine is accompanied or replaced by anserine, its methyl homologue. This Eggleton[1] has shown to have a $pK' = 7{\cdot}1$, so that its buffering range is little different from that of carnosine. Unfortunately, there is not at present an accepted routine method for the determination of anserine.

Proportion of buffering borne by carnosine in muscles of different species

The carnosine, orthophosphate, and total phosphorus[2] contents of the muscles of a number of species have been determined. The method used for carnosine was that of Eggleton & Eggleton [1933], for inorganic and total phosphorus that of King [1932]. In Table II the

Table II

		P.c. of muscle				P.c. of buffering due to				
Species	Muscle	Ortho-P	Carnosine	Lactic acid	Total buffering pH 6–7	Protein	Phosphate	Carnosine	Lactic acid	Unidentified (possibly anserine)
Horse	Thigh	0·13	0·54	0·94	6·3	45·0	32·0	20·0	1·0	2·0
Ox	Shin	0·14	0·35	0·88	5·3	34·0	44·0	16·0	1·0	5·0
	Thigh	0·15	0·39	0·96	5·65	27·5	46·0	18·0	1·0	9·5
Pig	Psoas	0·17	0·25	0·81	5·8	31·0	47·0	10·5	1·0	10·5
		0·16	0·28	0·90	6·15	37·0	40·0	11·0	1·0	11·0
	,,	0·14	0·27	0·90	5·5	48·0	44·0	11·0	1·0	−4·0
	,,	0·10	0·41	1·20	5·35	45·0	32·0	17·0	1·5	4·5
Sheep	Thigh	0·13	0·14	0·74	5·3	40·0	42·0	6·5	1·0	10·5
	Loin	0·11	0·13	0·68	5·3	45·0	37·0	6·0	1·0	11·0
Dog	Thigh	0·13	0·08	0·75	4·9	35·0	47·0	4·0	1·0	13·0
Rabbit	Long. dorsi	0·15	0·26	1·17	6·2	31·0	40·0	10·0	1·0	18·0
	Thigh	0·14	0·11	0·93	5·75	40·0	40·0	5·0	1·0	14·0
Rat	Mixed	0·19	0·07	0·87	5·3	26·5	55·0	3·0	1·0	14·5
	,,	0·19	0·06	0·74	4·9	26·0	61·0	3·0	1·0	9·0
	,,	0·19	0·07	0·99	5·3	31·0	57·5	3·0	1·0	7·5
Mean of 3 rats	Mixed	0·18	0·06	0·9	4·85	31·0	57·5	3·0	1·0	7·5
Ditto (trained)	,,	0·15	0·05	0·9	5·05	40·0	47·0	2·0	1·0	10·0
Chicken	Leg	0·13	0·05	0·71	4·8	44·0	42·0	2·0	1·0	11·0

results of these determinations are summarized, and the proportion of the buffering of the muscle between pH 6 and 7·5 attributable to protein, phosphate, and carnosine respectively is calculated from this data and included in the table. It is to be noted that muscle taken from a freshly killed animal and treated with chloroform vapour has practically no phosphate other than orthophosphate. The small difference between

[1] Private communication.

[2] Total P was found on the average to be only about 3 p.c. higher than ortho-P. For the sake of brevity the values are omitted from the table.

orthophosphate and total phosphate is probably due to hexose monophosphate, but since the relevant pK' values of the hexose phosphoric esters also lie between 6 and 7 this small amount of esterified phosphate can be regarded as orthophosphate without appreciable error.

The buffering ultimately unaccounted for is at a minimum of 5 p.c. in the horse and ox, and at a maximum of 20 p.c. in the rabbit. The buffering due to carnosine is at a maximum of 20 p.c. in the horse, ox and pig and at a minimum of 2 p.c. in the rat. The values for anserine recorded in the literature are large enough to fill the gap in many cases. Some of these values are collected in Table III, together with some determinations of carnosine in the same species.

TABLE III

Muscle	P.c. carnosine	P.c. anserine	Observer
Horse	0·2–0·5	None	K., K.-A., W.
Ox	0·2–0·6	None	K., K.-A., W.
Pig	0·2–0·4	None	K.-A.
Dog	0·1	0·1	W.
Rabbit	0·05–0·2	0·15	W.
Fowl	0·05–0·15	0·1–0·4	K.-A., W.

K. = Kuen [1927], K.-A. = Kapeller-Adler and Haas [1934], W. = Wolff & Wilson [1935].

Variation in buffering of muscle

As the figures in Table II show, there is a fairly wide variation between animals of different species, the lowest recorded being 4·8 for the rat, the highest 6·3 for the horse. These differences are of considerable practical importance, for they are large enough to determine the fate of a carcase. In the case, for instance, of two muscles each containing 0·7 p.c. of glycogen, in which 8 milliequivalents p.c. of lactic acid would be produced, the muscle with a buffering power of 4·8 would reach pH 5·9, whilst that with a buffering power of 6·2 would reach only pH 6·3 and would be in danger of undergoing rapid putrefaction or of becoming "bone tainted". Unfortunately there does not seem to be any simple means of lowering the buffering power of muscle without at the same time lowering its glycogen reserves. The highest gross variation is in the phosphate fraction (from 1·6 to 3·0) corresponding to a variation of 0·1–0·2 p.c. of phosphorus. Individuals of any one species appear to vary within rather narrower limits. A large number of albino rats have been examined, and irrespective of age and treatment the buffering from pH 6 to 7 fell within the limits of 4·8–5·3.

With the possible exception of carnosine and anserine, none of the buffer constituents appears to be readily variable at the will of the

experimenter. Hunter [1925] showed that a short period of starvation caused a rapid loss of carnosine from the muscles in cats, and that it was readily restored by feeding with meat. However, starvation of longer duration than is already customary could hardly be recommended as a means to this end. Meat animals are, as a routine measure, starved for about 24 hr. before slaughter, yet the muscles of oxen and pigs so treated still contain the high concentration of carnosine recorded in Table II.

The position of carnosine and anserine in muscle physiology is difficult to interpret. No function has hitherto been allotted to these bodies, but it could certainly not be stated with any confidence that their role is specifically that of muscle buffers, for they vary in concentration both individually and collectively within such wide limits. Yet their buffering action cannot be ignored, for it may amount to 25 p.c. of the buffering at *p*H 7 of rigor muscle, or as much as 40 p.c. of the buffering of living muscle, in which most of the phosphate is esterified and in the form of substances which buffer comparatively weakly between *p*H 7 and 7·5. The *pK'* of phosphocreatine is 4·5, of hexose monophosphate 6·1 [Meyerhof, 1930]. Adenyl pyrophosphate with three phosphorus atoms in its molecule has only one hydrogen ion dissociating in this range [Lohmann, 1933].

The total buffering of the phosphate system in resting muscle works out at less than a quarter of that in rigor muscle. Taking, for instance, the distribution of phosphorus in resting aerobic frog's muscle as given by Eggleton & Eggleton [1929], the following estimate of the buffering at *p*H 7, in terms of the buffering of orthophosphate, can be made.

	P.c. of total P	Approximate buffering as orthophosphate
Orthophosphate	13–15	14
Phosphagen	49–53	Nil
Adenylpyrophosphate	31–32	10
Hexosephosphate	2–4	2
		Total 26

If similar values can be assumed for mammalian muscle with as much as 0·6 p.c. of carnosine it is evident that the buffering due to carnosine would be more than double that due to phosphates, and equal to that due to protein.

Buffering due to bicarbonate in living muscle

Fenn [1928] and Irving *et al.* [1932] have shown that the buffering due to bicarbonate *as such* at the *p*H of living muscle (estimated at 7·0–7·2) is quite small. In the frog, Fenn estimates that of a total

dB/dpH of 2·0 only 0·4 is attributable to HCO'_3; in the dog, Irving *et al.* find 0·3 of a total of about 4 attributable to carbonates. In virtue of *escape of* CO_2 from bicarbonate, the buffering power is of higher order, and represents at least half the total buffering power of the muscle. It should be noted, however, that buffering at this intensity can be maintained for only so long as bicarbonate is available, and calculation from the data of these authors shows that the mean content of 20 c.c. of CO_2 per 100 g. of resting muscle corresponds to no more than 0·01 *M* bicarbonate sufficient to accommodate only 0·09 p.c. of lactic acid. Fatigued muscle, according to Meyerhof [1922] contains practically no bound CO_2; as would be expected, the buffering by escape of CO_2 is exhausted by only a fraction of the lactic acid produced during prolonged contraction. While, therefore, the bicarbonate buffering is a powerful factor in stabilizing *p*H during the early (almost anærobic) stages of severe exercise, its effect rapidly weakens and, if lactic acid continues to be produced, eventually vanishes. The buffering then rests entirely on the protein, carnosine-anserine and phosphate fractions of the muscle.

Summary

60–75 p.c. of the buffering of mammalian skeletal muscle in rigor between *p*H 7·5 and 5·5 is due to non-protein substances. Orthophosphate accounts for about one-half of this non-protein buffering. The remainder is largely due to carnosine and anserine, which buffer strongly in this range with a maximum of buffering at about *p*H 7.

The possible importance of carnosine and anserine as buffers in living muscle is discussed.

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BLOOD HISTAMINE AND CARDIAC ACTIVITY

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The experiments with which this paper deals arose out of an enquiry into the possible relation to other aspects of cardiac metabolism of the production of histamine by the heart, which was reported by Anrep *et al.* [1936]. According to their experiments, the histamine production by the dog's heart in heart-lung preparations, which they found to be generally of the order of 0·5 γ/min., was increased by raising the arterial resistance, by adrenaline, and by asphyxial conditions. The experiments which we at first made gave no evidence of histamine production, but the histamine equivalents from the same samples of blood at times showed such large differences that definite conclusions could not be drawn. The method then available for the assay of blood histamine equivalents [Barsoum & Gaddum, 1935] was therefore subjected to a thorough examination, and a modified procedure evolved [Code, 1937].

Employing this method, the question of histamine production by the heart was again attacked. Our first series of experiments was carried out with the heart-oxygenator preparation [Evans *et al.* 1934], which was chosen because, according to the results of Anrep *et al.* considerable destruction of histamine took place in the lung when the heart-lung preparation was employed. It was anticipated that by using the heart-oxygenator preparation this constant destruction of histamine would be eliminated, thus ensuring such an accumulation of histamine in the blood as would be easily recognizable. It was thought that such an accumulation of histamine in the blood might incidentally throw light on certain differences between the behaviour of the heart in the heart-lung, as compared with the heart-oxygenator, preparation.

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It will be seen from what follows that our expectations with regard to histamine production were not fulfilled, and as we were at a loss to understand the reason for this discrepancy between our experiments and those of Anrep *et al.* we turned to the heart-lung preparation. Again, as will be seen, we were unable to secure evidence of an increase in the histamine equivalent of the cardiac venous, as compared with the arterial, blood.

Methods

The heart of the dog was used throughout.

All the methods employed have already been described elsewhere. Defibrinated blood was used in the heart-oxygenator circuit prepared as described by Evans *et al.* [1934], with modifications by Bogue & Gregory [1937]. In this preparation the auricles are supplied separately with blood from a common reservoir, and the outputs of the right and left ventricles can be separately collected and measured. When the inflow from the reservoir to the right auricle is closed, the output of the right ventricle consists entirely and solely of the cardiac venous return. Samples of this venous blood were collected simultaneously with samples of arterial blood from the left ventricle output. The usual procedure after collecting the samples was to measure off 5 c.c. portions which were then run into 7·5 c.c. of 10 p.c. trichloroacetic acid. In some instances blood was drawn direct from the coronary sinus into the acid, without in any way altering the result.

Heart-lung preparations were made in the usual way and coronary blood drawn off from the sinus by a Morawitz cannula. This was so connected as to return the sinus blood into the cannula in the superior vena cava, and by the insertion of a T-tube it was possible at any time to divert this blood to measure the coronary blood flow and take samples for comparison with simultaneous samples drawn from the aortic output.

Adrenaline administration was effected by running a first dose of adrenaline (Ciba, 1/10,000) solution directly into the venous reservoir, followed by continuous infusion at a suitable rate of 1/10,000 adrenaline solution.

Oxygen lack was produced by rapidly ventilating the oxygenator, or lungs, with a mixture of nitrogen 95 p.c. and carbon dioxide 5 p.c. For periods of low cardiac work, the arterial pressure was kept at about 40–60 mm. Hg, and the output small. For high work the arterial pressure was raised to about 120 mm. Hg or more, with or without suitable alterations in output. In some of the experiments with the heart-oxygenator preparation the output spontaneously changed with altera-

tions of arterial pressure and this was usually left uncompensated. In all cases the venous pressure in the inferior vena cava was recorded and the maintenance of a low venous pressure was taken as an indication that the heart was in good condition.

Extracts of blood were prepared, as a routine, by a modification of the Barsoum-Gaddum method [Code, 1937]. All estimations were carried out in duplicate or triplicate, and in some cases even in quadruplicate. Throughout one experiment, duplicate extracts were prepared by the Barsoum-Gaddum method and by the modified method. All samples were assayed by the guinea-pig's ileum suspended in 2–3 c.c. of Tyrode's solution containing atropine sulphate (1×10^{-8}). The activity of the extracts was expressed as equivalent to histamine base.[1]

With extracts prepared by the modified method, histamine equivalents below 0·01γ/c.c. have been shown to be of somewhat doubtful significance and to be subject to considerable variation [Code, 1937]. Although in this study values below 0·01γ/c.c. have been assayed and are included in the tables, this has only been done to indicate the *approximate* range of differences which occurred between samples; for we are of the opinion that activity of this order cannot conclusively be attributed to the presence of histamine in the blood.

During the performance of a long series of assays on blood of very small histamine equivalents (0·01γ/c.c. or less) on the same piece of gut, it was frequently found that the intestine became reduced and erratic in sensitivity, but its sensitivity was restored by a somewhat larger dose of histamine. On the other hand, when definite histamine activity was present in the extracts the sensitivity usually became slightly augmented after a few estimations had been performed.

It became important in the investigation to ascertain whether or not quantities of histamine of the order of 0·01–0·04γ/c.c. added to blood could be recovered in extracts prepared by the modified method. Such quantities of histamine were therefore added to blood of low initial histamine-like activity, and the recovery in the extracts was satisfactorily effected (86–102 p.c.).

Since in most of our experiments the blood histamine equivalent was of the order of 0·01–0·02γ histamine base per c.c., it is of prime importance to recognize the limits of error attendant upon histamine assay. Suppose, for example, that it is possible to distinguish between 0·01 and

[1] Anrep *et al.* [1936, p. 432] stated that their results were expressed in terms of histamine acid phosphate. We are uncertain whether this was actually the case, but if so their result should, for comparison with ours, be divided by three.

0·02γ/c.c., such a change from the lower to the higher content, in passing through the heart, supposing the blood flow to be 50 c.c./min., would mean a histamine production of 0·5γ/min. This rate of production lies somewhere about the average found by Anrep *et al.*, though in many of their experiments it was much greater.

In our experience such a difference in the histamine content of blood is easily detectable. We believe, however, that the method of assay does not permit the consistent and accurate detection of differences smaller than ±0·005γ/c.c., and this probably represents the limit, using duplicate estimations. Under the conditions of blood flow mentioned above this corresponds to a possible error in the estimation of the histamine production of 0·25γ/min.

In view of the statement by Anrep and his pupils, that the histamine production was usually between 0·1 and 1·5γ/min., generally about 0·5γ/min. but might on occasion rise to even 2γ/min., we expected, in spite of the acknowledged limits of the method, the regular detection of some arterio-venous difference in the blood histamine equivalent.

Results

(1) *Experiments with the heart-oxygenator preparation*

In these experiments, whatever the conditions, we never detected a difference between the histamine equivalent of arterial and coronary blood which was greater than the difference between duplicates performed on the same sample of blood, nor did the usually trivial histamine equivalent of the arterial blood show any significant changes throughout an experiment (Table I).

In Exp. 1 the effect of raising the arterial pressure from 42 to 123 mm. Hg, of adding adrenaline (0·19 mg. in 10 min.) and of 15 min. progressive oxygen lack induced by ventilation with N_2/CO_2 mixture were successively tested. The oxygen deprivation was carried nearly to the point of failure (venous pressure 14 cm. H_2O) before sampling the blood. Exp. 2 was essentially similar except that the arterial pressure was raised to 135 and 140 mm. Hg, and the partial anærobiosis preceded the adrenaline period instead of following it.

In order to ascertain whether the introduction of a cannula into the coronary sinus might possibly cause damage leading to localized production of histamine, a heart-oxygenator preparation was made in the usual way and the effects of high arterial pressure, oxygen lack and adrenaline were tested as in the previous experiments. Then a Morawitz

TABLE I. Heart-oxygenator experiments

Min. after commencement	Aortic pressure mm. Hg	S. V. C. pressure cm. H_2O	Heart rate per min.	Blood flow, c.c./min.		Adrenaline given mg.	Duration of oxygen lack min.	Histamine equivalent γ/c.c. (mean of two)		Remarks
				Aorta	Coronary			Arterial	Coronary	
Exp. 1. Heart-oxygenator. Heart = 113 g. Blood vol. = 600 c.c.										
35	42	1·5	123	160	217	0	0	<0·01	0·01	
48	123	1·5	123	162	206	0	0	<0·01	<0·01	
108	123	0·5	240	103	333	0·19 mg. in 10 min.	0	<0·01	<0·01	
131	123	14·0	—	37	174	0	15	<0·01	<0·01	Oxygen lack by N_2 + 5 p.c. CO_2. Heart failing
Exp. 2. Heart-oxygenator. Heart = 117 g. Blood vol. = 950 c.c.										
27	55	1·0	105	560	62	0	0	0·009	0·009	
46	135	4·0	110	180	238	0	0	0·009	0·009	
60	77	3·5	113	400	170	0	0	0·009	0·009	
74	77	8·5	—	248	150	0	13	0·009	0·01	By N_2/CO_2
103	77	6·5	111	248	151	0	0	0·008	0·008	
125	140	5·5	231	153	390	1·4 mg. in 19 min.	0	0·013	0·009	
Exp. 3. Heart-oxygenator, followed by Morawitz cannula. Heart = 90 g.										
29	60	0·5	140	250	195	—	—	0·008	0·010	
60	60	19·0	137	80	116 (failing)	—	25	0·008	0·008	Failure commencing
72	63	2·0	132	340	200	—	—	0·008	0·009	
105	110	7·0	128	290	300	—	—	0·009	0·009	
128	133	0·0	261	290	567	0·31 mg. in 20 min.	—	0·011	0·013	
Morawitz cannula inserted at 158 min. and samples for analysis taken therewith										
188	130	1·5	256	300	440	0·21 mg. in 17 min.	—	0·01	0·011	
202	130	7·0	—	48	V. slow	0·19 mg. in 14 min.	14	0·009	0·009	Failing

* The coronary flow given is the entire flow returning to the right heart. When the Morawitz cannula was inserted the flow collected by it was added to that returned by the pulmonary artery and representing the remaining coronary drainage. The coronary sinus flow ranged from 0·71 to 0·77 of the whole coronary flow.

cannula was introduced into the coronary sinus and samples of blood for analysis were collected from this, first during a period in which high arterial pressure was combined with adrenaline administration, and then for a period in which high pressure, adrenaline and oxygen lack were combined. Again there was no evidence of histamine production (Exp. 3, Table I).

If histamine is not destroyed in the blood, it would be expected that the blood of a heart-oxygenator preparation should, if the heart is producing histamine, at say, 1γ/min., accumulate it at the rate of 60γ/hr. With 600 c.c. of blood in the circuit, this should mean a rise of blood histamine of the order of 0·1γ/c.c./hr.; an amount which, even acknowledging the lack of delicacy of our method, could not fail to be recognized. The results (Table I) proved that no such accumulation occurred during 2–3 hr.

Thus in the heart-oxygenator preparation, no production of histamine was detected. It was unlikely that had histamine been produced by the heart we could have failed to detect it. Therefore, we attempted to reproduce as closely as possible the conditions of Anrep *et al.* in experiments on the heart-lung preparation.

(2) *Experiments with the heart-lung preparation*

Histamine equivalent estimations have been made on the arterial and coronary sinus blood of heart-lung preparations as shown in Table II.

In one experiment (Exp. 7, Table III) extracts were prepared from the same blood both by the Barsoum-Gaddum method and by the modified procedure. The histamine equivalents obtained by the modified method were slightly higher than those given by the Barsoum-Gaddum method.

Assay of these parallel extracts showed that with either method there was no evidence of production of histamine by the heart. In the remaining experiments of the series also (Table II) the differences in histamine equivalent between the arterial and coronary bloods were, with one exception, not greater than the differences between duplicate estimations performed on the same sample of blood. In this one exceptional case (Exp. 6, Table II), when the pressure was raised to 120 mm. Hg, the estimations indicated a production of 0·005γ/c.c. which, when multiplied by the coronary flow, was equivalent to a production of 0·33γ/min. by the heart. That the isolated occurrence of such differences is of doubtful value is shown by a similar calculation in the subsequent

TABLE II. Heart-lung experiments

Min. after commencement	Aortic pressure mm. Hg	S. V. C. pressure cm. H_2O	Heart rate per min.	Blood flow, c.c./min.		Adrenaline given mg.	Duration of oxygen lack min.	Histamine equivalent γ/c.c. (mean of two)		Remarks
				Aorta	Coronary			Arterial	Coronary	
Exp. 4. Heart-lung preparation. Heart = 168 g. (œdematous)										
0	60	1·0	126	540	<10	—	—	0·006	—	
37	60	1·0	126	540	14	—	—	0·006	0·008	
70	125	2·5	133	720	32	—	—	<0·005	0·007	
107	125	2·0	218	580	130	0·29 mg. in 10 min.	—	0·005	0·006	
125	125	17·5	—	164	417	—	15	<0·005	<0·005	Heart failing
Exp. 5. Heart-lung preparation. Heart = 146 g.										
31	58	1·0	150	638	18	—	—	0·0	<0·005	
50	126	1·0	169	610	34	—	—	0·0	0·0	
80	126	1·0	227	578	215	0·29 mg. in 19 min.	—	0·0	0·0–0·007	
103	126	2·5	227	345	350	0·36 mg. in 18 min.	18	0·0–<0·005	0·0–<0·005	
Exp. 6. Heart-lung preparation. Heart = 80 g.										
32	67	1·0	160	570	22	0	0	0·018	0·020	Samples collected
38	120	1·0	160	—	66	0	0	0·01	0·015	absolutely simul-
50	70	0·5	235	477	66	0·1	0	0·015	0·010	taneously in all
75	225	4·0	230	194	352	0·1	0	<0·01	<0·01	cases
78	225	4·0	230	194	362	—	0	—	<0·01	

TABLE III. Estimation of histamine by two methods. Exp. 7. Heart-lung preparation. Heart = 195 g.

Min. after commencement	Aortic pressure mm. Hg	S. V. C. pressure cm. H_2O	Heart rate per min.	Blood flow c.c./min.		Adrenaline given mg.	Duration of asphyxia min.	Histamine equivalent (Code) γ/c.c. (mean of two)		Histamine equivalent by Barsoum & Gaddum's method γ/c.c.	
				Aorta	Coronary			Arterial	Coronary	Arterial	Coronary
18	63	1·0	128	518	25	—	—	0·022	0·024	0·018	0·018
45	147	2·5	135	1035	99	—	—	0·022	0·023	0·010	0·015
62	130	5·0	138	500	119	—	25	0·020	0·023	0·014	0·016
100	107	0·5	189	518	152	0·2	—	0·023	0·021	0·015	0·017

period, where adrenaline was given (0·005γ/c.c. or 0·33γ/min. *removed* by the heart).

There were apparently two possible causes for our failure to demonstrate histamine production by the heart. The first was that the routine method for the estimation of the histamine equivalent was not sufficiently sensitive to detect an increase of histamine in the coronary blood. This has already been discussed. The second was that the histamine produced by the heart was rapidly disappearing from the blood. Experiments were therefore performed to test this possibility.

(3) *Addition of histamine to the heart-lung and heart-oxygenator preparations*

In one experiment by the addition of 50γ of histamine (base), the histamine equivalent of the circulating blood in a heart-lung preparation was raised from 0·005 to 0·026γ/c.c. and again to 0·04γ/c.c. by a further addition of 50γ histamine. Finally, 100γ histamine was added, raising the histamine equivalent to 0·07γ/c.c. During the subsequent 30 min. this value fell to 0·034γ/c.c. (Exp. 8, Table IV). Calculation showed that histamine was being lost from the blood at a rate proportional to its concentration. Presumably this was by destruction of histamine in the lungs [Best & McHenry, 1930].

In a similar experiment (Exp. 9, Table IV) a heart-lung circuit was converted to a heart-oxygenator preparation. Additions of similar quantities of histamine indicated a slower rate of destruction of histamine in the latter, but nevertheless there was some loss, viz. a reduction of 0·02γ/c.c. in 43 min. The rate of loss was not reduced when adrenaline was added. Control samples kept at 37° C. in tonometers for comparable periods showed a loss of about 0·01γ/c.c. in that time, which indicated that the heart was not wholly responsible for the slow loss of histamine from this circuit. It would, however, appear that had the heart been *producing* histamine at, say only 0·5γ/min., i.e. 21·5γ in

TABLE IV. Addition of histamine

Min. after commencement	Aortic pressure mm. Hg	S. V. J. pressure cm. H_2O	Heart rate per min.	Blood flow, c.c./min.		Adrenaline given mg.	Duration of oxygen lack min.	Histamine equivalent γ/c.c. (mean of two)		Remarks
				Aorta	Coronary			Arterial	Coronary	
Exp. 8. Heart-lung preparation. Heart = 120 g. Blood volume = 700 c.c.										
120	100	2	130	490	59	—	—	0·005	—	
123	—	—	—	—	—	—	—	—	—	Add 50γ histamine
125	100	—	—	—	50	—	—	—	—	
130	100	2	130	490	50	—	—	0·026	—	
131	—	—	—	—	—	—	—	—	—	Add 50γ histamine
135	—	—	—	490	74	—	—	—	—	
140	—	2	—	—	—	—	—	0·041	—	
141	—	—	—	—	—	—	—	—	—	Add 100γ histamine
150	100	2·5	130	490	60	—	—	0·070	—	
152	100	—	—	—	54	—	—	—	—	
182	—	4·5	—	—	—	—	—	0·034	—	
Exp. 9. Heart-lung and heart-oxygenator preparation. Heart = 205 g.										
Heart-lung. Blood vol. 950 c.c.										
20	90	3·0	150	350	—	—	—	0·024	—	
25	—	—	—	—	—	—	—	—	—	Add 100γ histamine
35	90	3·0	150	353	—	—	—	0·063	—	
57	90	—	—	—	—	—	—	0·046	—	
Heart-oxygenator. Blood vol. 670 c.c.										
88	95	—	—	313	132	—	—	—	—	
89	—	8·5	—	—	—	—	—	—	—	Add 50γ histamine
100	105	10·0	129	—	136	—	—	0·044	—	
120	105	10·5	126	160	150	—	—	0·034	0·036	
143	105	1·0	200	226	281	0·1	—	0·024	0·024	

43 min., this should have *raised* the level by about 0·02–0·03γ/c.c., so that histamine would have accumulated in determinable quantities in the circulating blood.

Discussion

In their various experiments Anrep *et al.* found the cardiac venous blood to have a histamine activity ranging from two to nine times as great as that of the arterial blood, while in only about 2 p.c. of the 250 samples collected by them did the difference between the arterial and the coronary venous blood lie within the limits of accuracy of the method.

Although it is evident that there must have been some fundamental difference between the way in which their experiments and our own were carried out, we cannot imagine wherein that difference may lie. Their finding that hearts in weak condition do not show the phenomenon is inadequate to explain our failure, in spite of our thorough familiarity with heart-lung technique, ever to secure a heart in such a state as to show a production of histamine. The condition of the heart is, we believe, best judged by the venous pressure in the inferior vena cava or right auricle, and this we invariably recorded. At the commencement of an experiment it lies near to zero, but owing to the difficulty of assessing the zero level, we have arbitrarily called the initial very low pressure +1 cm. H_2O. Many of our determinations were made at times when the venous pressure had shown no increase, or only moderate increases, on raising the arterial pressure. Ventilation of the lungs with air gave the same results as when oxygen with 5 p.c. CO_2 was employed.

The high histamine content of the blood of their dogs, though perhaps explicable on the ground that their results were expressed, as they state them to have been, as the acid phosphate and not as the base, could also provide no satisfactory explanation for a two- to ninefold increase of concentration in the venous blood. Nor could an undue faith on their part, or doubt on our own, regarding the limits of accuracy of the method of histamine assay, explain their almost unfailing success, or our unvaried failure, in detecting an increase of histamine in the coronary blood.

The high coronary flows which were obtained with the heart-oxygenator preparations as compared with the heart-lung preparations might certainly have reduced any arterio-venous differences, but we find it hard to believe that such reduction would be so great as to bring the difference to within the limits of error of the assay.

SUMMARY

No evidence for the production of histamine by the dog's heart could be found, either in heart-oxygenator or in heart-lung preparations.

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THE OSMOTIC PRESSURE OF THE AQUEOUS HUMOUR IN THE NORMAL AND GLAUCOMATOUS EYE

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The osmotic relationship between blood and aqueous humour is of great importance when considering the origin and nature of intra-ocular fluid. One of us [Duke-Elder, 1927] determined the osmotic pressure of the aqueous humour by direct measurement in association with physico-chemical analyses, and concluded that the molecular concentration was less than that of the blood by an amount compatible with the hypothesis that this fluid might be formed by a process of dialysation. These results seemed to be verified in a later investigation using the much more accurate technique of the vapour-pressure method of A. V. Hill as modified by Baldes [Benham *et al.* 1937]: in this investigation anæsthetized cats were used, and the results showed that the osmotic pressure of the aqueous humour was less than that of the corresponding serum. The differences found were of the order necessitated by theory, for the osmotic pressure of the former was less than that of the latter by the osmotic pressure of the plasma proteins (about 27 mm. Hg) plus the small pressure difference existing by virtue of the unequal distribution of electrolytes (about 5 mm. Hg), that is, by a difference corresponding to about 0·0018 *M* NaCl.

The present investigation was undertaken with a view to studying any changes there might be in the molar concentration of the intra-ocular fluids in the pathologically raised tension of primary glaucoma in man. The results showed a reversal of the relationship previously found in animals, but any fundamental pathological significance attached to this observation was immediately discounted by the discovery that in

human controls in whom the intra-ocular pressure was normal, the osmotic pressure of the aqueous humour was again higher than that of its corresponding serum. As an alternative to the unlikely assumption that the aqueous humour in man was radically different from that of the animals investigated (rabbits and cats), it appeared to us worth considering whether the fact that the animal experiments were done under general anæsthesia and the human experiments under local anæsthesia had any bearing on the discrepancy. The work of Margaria [1930], for example, showed that the effect of forced exercise raised the osmotic pressure of the blood by very large amounts (up to 500 mm. Hg), and it was considered possible that the muscular movements associated with anæsthesia as well as the effect of ether on the acid-base equilibrium might have reversed the relationship between the two fluids by artificially raising the molar concentration of the blood. This surmise proved to be correct. Dogs were chosen as being the most amenable animal to train to permit the withdrawal of aqueous humour under a local anæsthetic, and it was found in these animals that under local anæsthesia the molar concentration of the aqueous humour was higher than that of the blood, but that under ether anæsthesia the osmotic pressure of the blood had risen by an amount which reversed this relationship. The matter was further verified in a cat anæsthetized with 150 mg. of sodium amytal injected intraperitoneally; for under this anæsthetic (the administration of which involves no violent muscular movements) the same relationship was found, a result the opposite of that previously published with regard to this animal with ether anæsthesia.

Experimental

(*a*) *Experiments on man*

Samples of blood and aqueous humour were obtained from patients with chronic glaucoma and from a control group of non-glaucomatous subjects. In all cases factors such as exercise or the ingestion of food or fluids, which would be likely to affect the blood-aqueous equilibrium, were carefully controlled. A sample of aqueous humour, about 0·1 ml., was obtained by passing a fine needle obliquely through the limbus of the cocainized eye. Blood was collected anærobically and without stasis, while every precaution was taken to prevent access of air to the blood until the serum had been separated, and in each case the sample was equilibrated with 5 % CO_2. It was withdrawn from the antecubital vein after the temperature of the arm had been brought to 45° C. or,

where possible, from a vein on the back of the hand, where the oxygen content of the blood approximates that of arterial blood even more closely. The osmotic concentration, as has been said, was determined by the thermo-electrical method of A. V. Hill as modified by Baldes.

The results are given in Tables I and II. They comprise a study of fourteen cases of chronic primary glaucoma and seven non-glaucomatous subjects selected as controls. The values are represented in terms of molar sodium chloride.

In Table I are given the osmotic pressures of the blood and aqueous humour of fourteen cases of chronic primary glaucoma and seven control cases. It will be seen that there is no significant difference between the glaucomatous cases and the controls and that although there is a variation in absolute values between individual cases, in all except one the osmotic pressure of the aqueous humour exceeded that of the blood. The twenty-one cases investigated showed a wide range of intra-ocular pressure ranging from 18 to 70 mm. Hg, and consequently it is obvious that pathologically raised tension in the eye is not a simple function of the osmotic balance between the aqueous humour and the blood. In three cases it was possible to obtain more than one sample of blood and aqueous humour, so that the osmotic pressure of aqueous humour could be studied over a period of time. Detailed data respecting these cases is given in Table II.

TABLE I

Case no.	Molarity of aqueous humour	Molarity of serum	Difference
1. Chronic primary glaucoma	0·1597	0·1520	+0·0077
2. " "	0·1618	0·1581	+0·0037
3. " "	0·1651	0·1613	+0·0038
4. " "	0·1624	0·1598	+0·0026
5. " "	0·1584	0·1541	+0·0043
6. " "	0·1647	0·1626	+0·0021
7. " "	0·1592	0·1584	+0·0008
8. " "	0·1589	0·1586	+0·0003
9. " "	0·1584	0·1561	+0·0023
10. " "	0·1571	0·1547	+0·0024
11. " "	0·1583	0·1540	+0·0043
12. " "	0·1574	0·1539	+0·0040
13. " "	0·1608	0·1544	+0·0064
14. " "	0·1618	0·1567	+0·0051
Mean of 14 glaucoma cases	0·1602	0·1566	+0·0036
15. Senile cataract	0·1598	0·1604	−0·0006
16. Detached retina	0·1603	0·1563	+0·0040
17. Aphakia	0·1560	0·1527	+0·0033
18. Sarcoma of choroid	0·1614	0·1543	+0·0071
19. Aphakia	0·1582	0·1507	+0·0075
20. Congenital cataract	0·1586	0·1530	+0·0056
21. Aphakia	0·1606	0·1551	+0·0055
Mean of 7 control cases	0·1593	0·1545	+0·0048

TABLE II

Case 12 (chronic primary glaucoma)	Molarity of aqueous humour	Molarity of serum
(i) Initial withdrawal	0·1579	0·1539
(ii) Reformed aqueous humour taken 15 min. later	0·1568	—
(iii) Aqueous humour and blood withdrawn 24 hr. later	0·1596	0·1530
Case 13 (chronic primary glaucoma)		
(i) Initial withdrawal	0·1608	0·1544
(ii) Aqueous humour withdrawn 24 hr. later	0·1614	—
(iii) Aqueous humour and blood withdrawn 5 days later	0·1591	0·1534
Case 18 (sarcoma of choroid)		
(i) Initial withdrawal	0·1614	0·1543
(ii) Aqueous humour and blood withdrawn 24 hr. later	0·1602	0·1549

(*b*) *Experiments on animals*

Experiments were performed on dogs using a similar technique. Table III gives the results of measurements in locally anæsthetized dogs, and also the comparative results where aqueous humour from the other

TABLE III

	Under local anæsthesia			Under ether anæsthesia			
	Molarity of aqueous humour	Molarity of serum	Difference aqueous humour – serum		Molarity of aqueous humour	Molarity of serum	Difference aqueous humour – serum
Dog 1	0·1674	0·1674	0		—	—	—
14 days later	0·1650	0·1661	–0·0011		—	—	—
Dog 2	0·1619	0·1600	+0·0019		—	—	—
7 days later	0·1651	0·1621	+0·0030		—	—	—
Dog 3	0·1662	0·1604	+0·0058		—	—	—
Dog 4	0·1689	0·1608	+0·0081		—	—	—
Dog 5	0·1662	0·1610	+0·0052		—	—	—
6 days later	0·1652	0·1626	+0·0026	(*)	0·1648	0·1662	–0·0014
Dog 6	0·1669	0·1568	+0·0101	(*)	0·1647	0·1651	–0·0004
Dog 7	0·1629	0·1598	+0·0031	(*)	0·1646	0·1708	–0·0062
Dog 8	0·1666	0·1581	+0·0085	(*)	0·1647	0·1685	–0·0038
Dog 9	—	—	—	(*)	0·1640	0·1628	+0·0017
				(1 hr.)	0·1709	0·1717	–0·0008
				(2½ hr.)†	0·1738	0·1744	–0·0006
Dog 10	0·1678	0·1589	+0·0089	(2½ hr.)	0·1719	0·1696	+0·0021
				(4½ hr.)†	0·1743	0·1693	+0·0050
Dog 11	0·1646	0·1611	+0·0035	(1 hr.)	0·1685	0·1692	–0·0007
Dog 12	—	—	—	(2 hr.)	0·1718	0·1688	+0·0030
				(4 hr.)	0·1726	0·1667	+0·0059
Dog 13	0·1604	0·1568	+0·0036	(2½ hr.)	0·1717	0·1726	–0·0009
Mean	0·1653	0·1608	+0·0045	(*)	0·1646	0·1666	–0·0020
Cat							
Sodium amytal anæsthesia	0·1755	0·1706	+0·0049		—	—	—

* Figures relate to samples taken immediately after anæsthesia.
† Denotes sample of aqueous humour taken from same eye as first sample.

eye and a second sample of blood were withdrawn under ether anæsthesia administered immediately afterwards. It is seen that in the former the relationship between the molar concentration of the aqueous humour and the serum is the same as in the human experiments; while after ether anæsthesia the osmotic pressure of the blood is sent up to a high level so that this relationship is reversed. When, however, the anæsthesia is prolonged for a period of hours the molarity of the aqueous humour increases after a time lag. In dogs 10 and 12 after 4 hr. anæsthesia the difference between the osmotic pressure of blood and aqueous humour is in the same direction as in normal unanæsthetized animals. Thus after a time a concentration gradient is resumed.

Plasmoid intra-ocular fluid

After the initial sample of aqueous humour is taken an intra-ocular fluid which differs markedly from the normal aqueous humour, and resembles plasma, reforms quickly. In certain cases the osmotic pressure of this reformed or plasmoid aqueous humour was investigated. Thus in dogs nos. 3, 4 and 5, whereas the osmotic pressure of initial aqueous humour was 0·1662, 0·1669 and 0·1629 *M*, that of the intra-ocular fluid reformed after 15 min. was found to be 0·1648, 0·1643 and 0·1613 *M*, a change in all cases in the direction of the values obtained for the corresponding serum. A similar result was obtained in man, as shown in Table II, case 12 (ii).

That after a considerable period of time, normal conditions are resumed, and the initial relationship between aqueous humour and blood obtains, is shown in man (Table II, cases 12, 13 and 18) and in dogs (Table III, nos. 1, 2 and 3). The significant fact emerges that whereas the immediately reformed intra-ocular fluid appears to be formed directly from the plasma, and tends to resemble it in osmotic pressure, a different process is involved in the production of normal aqueous humour.

Summary

1. The molar concentration of the aqueous humour of man, the dog and the cat is higher than that of the corresponding serum by an amount averaging 0·004 *M* NaCl.

2. The immediate effect of ether anæsthesia is to raise the molar concentration of the blood by as much as 0·01 *M* NaCl, so that the normal relationship between blood and aqueous humour is reversed.

3. Pathological intra-ocular tension is not a simple function of the osmotic balance between the blood and aqueous humour.

4. Owing to the osmotic relationship between the intra-ocular fluid and the blood, it is impossible that the former is a simple dialysate.

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EXPERIMENTAL TRAUMATIC SHOCK

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The primary feature of traumatic shock is the low blood pressure and in recent years this has mainly been attributed to oligæmia rather than vasomotor paralysis. This oligæmia is held by many observers to be the result of a localized extravasation of blood and plasma into the traumatized zone and adjacent soft tissues—Smith [1927–8], Blalock [1930], Parsons & Phemister [1930], Freelander & Lenhart [1932] and Holt & Macdonald [1934, 1935], and not due to a generalized filtration out of the vessels as visualized by Malcolm [1909]. Starling [1918] held the view that the blood tended to be stagnated in the vascular bed of the voluntary muscles. More recently it has been suggested that the effect of this loss of blood volume at the site of trauma is reinforced by a discharge of nerve impulses from the traumatized area—Simonart [1930], O'Shaughnessy & Slome [1934–5, 1935]. The toxæmic theory, introduced by Quénu [1918], by Cannon & Bayliss [1919], and supported by numerous other observers in the Great War to explain the hypotensive effects of trauma, has not found favour in recent years although still upheld by many of its original exponents. The observations of Barsoum & Gaddum [1935–6] on the rise of blood histamine in cases of extensive cutaneous lesions at about the time when secondary shock is liable to develop are of interest, although not necessarily bearing on the cause of mechanical shock.

Cannon & Bayliss appreciated the fact that local loss of blood and fluid into the damaged tissues, even when slight, might be a contributory factor, although their method of comparative weighings failed to give a true picture of the extent of the extravasation and hæmorrhage. They also considered that there was no essential relationship between the production of shock and an excessive stimulation of the nervous system, but here their evidence is unconvincing. Parsons & Phemister [1930]

found that the hammering of denervated limbs of dogs resulted in low blood pressures similar to that produced in the animals whose limbs were not denervated. Freelander & Lenhart [1932] also considered that the nervous factor was unimportant, but the evidence is equivocal. As the result of their observations on cats anæsthetized with chloralose, O'Shaughnessy & Slome [1934–5] uphold the view that a major factor in the onset and maintenance of shock is a continuous centripetal discharge of nerve impulses. They found that spinal anæsthesia had a most favourable influence in delaying and even preventing the onset of shock. Holt & Macdonald [1934, 1935], on the other hand, have found no evidence which supports this view that nociceptive nervous stimuli from the injured tissues dominate the picture in dogs anæsthetized with sodium barbitone.

Against the toxæmic theory are these numerous experiments which have shown that blood from a traumatized extremity, when collected during the shock-producing period and when injected into a normal animal, fails to produce a depressor effect—Smith [1927–8], Blalock [1930], Parsons & Phemister [1930], Schneider [1930], Holt & Macdonald [1934], O'Shaughnessy & Slome [1934–5] and Dragstedt & Mead [1937]. Parsons & Phemister also found that the yield of depressor substances from the traumatized muscles of an animal which had developed shock was no higher than that derived from an equivalent weight of normal muscle.

The second main criticism levelled against traumatic toxæmia is that the loss of fluid into the damaged area is sufficient in itself to account for even half of the initial total calculated volume of blood—Blalock [1930], Parsons & Phemister [1930], Freelander & Lenhart [1932] and Holt & Macdonald [1934]. Brooks & Blalock [1934] have also shown that a continued low blood pressure of approximately 70 mm. due to the gradual withdrawal of blood may in itself produce death of the animal within 1 hr. Transfusion was without benefit if the blood pressure had remained at a low level for several hours.

Although the evidence thus weighs against the liberation of a toxin into the circulation, there still remains to be explained the reduction in blood volume.

The present investigation is an effort to assess the parts played by fluid loss, nervous impulses and toxæmia in the onset of traumatic shock in the deeply anæsthetized experimental animal.

Experimental

The experiments in this series were all performed on anæsthetized cats (ether or nembutal), and for descriptive purposes are divided into two groups. Blood-pressure records were taken by means of carotid cannulæ. Trauma took the form of crushing or repeated hammering with a steel bar of the thigh muscles of one limb with or without fracture of one or more of the long bones.

Group 1. Some animals were anæsthetized with intratracheal ether, others with nembutal during the period of trauma. One received chlorazol fast pink as anticoagulant. Trauma to the thigh muscles, but without fracture of bone, led to the death of the five animals in this series in from 12 to 99 min. following its application (mean 47 min.). A second trauma was inflicted on two of the animals shortly after the first. In these two animals the first period of trauma induced a rapid fall of blood pressure succeeded by a rapid rise to normal level. The second trauma induced the typical slow and progressive fall in pressure. The final stages in all these animals was marked by a sudden fall in blood pressure. The survival period was comparable to that of the donors in the second series of cross-circulation experiments.

Group 2. Cross-circulation experiments (Table I, Fig. 1). Donor and recipient each received 0·065 g. nembutal subcutaneously, and this almost invariably induced surgical anæsthesia within half an hour. In the course of the experiment additional doses of 0·015–0·020 g. were given as required. As a rule only two additions were necessary.

Preparation of recipient. Trachetomy was performed and a cannula was inserted into the trachea. The right limb was then prepared so that it could be rendered anæmic, somewhat in the manner described by O'Shaughnessy and Slome. An incision was made from the last rib to the crest of the ilium. The muscles were divided and the peritoneum was stripped inwards off the posterior abdominal wall until the abdominal aorta and inferior vena cava were exposed. Thus the posterior abdominal wall was exposed extraperitoneally. The vessels were looped with thread but not tied off. The aorta was traced downwards until the right external iliac artery was exposed, and a loop of thread was also passed round this vessel. The middle sacral artery was picked up at its origin at the bifurcation of the aorta into the external iliac arteries, and the middle sacral artery and vein were included in a single loop. The inferior vena cava was traced downwards to the commencement of the right common iliac vein, which was included in a loop of thread. The free ends of all these ligatures

were withdrawn through the wound, the muscles were stitched together, and the skin flaps approximated. The right femoral vessels were picked up and loose ligatures passed round each. It was believed that when all these vessels were ligated the right limb would be completely cut off from the general circulation.

Preparation of the donor. The femoral vessels of the left hindlimb were exposed and loosely ligated. Each animal was now given 0·1 g. chlorazol fast pink per kg. body weight dissolved in 10 ml. saline of such strength that the resultant solution was isotonic.

Establishment of cross-circulation. Right-angled cannulæ of small bore and short lengths of rubber tubing filled with citrate solution were used. The femoral artery and vein of the donor were clamped proximally and tied distally, and the cannulæ inserted proximally into the artery and vein. The rubber tubing was clamped and the clamps removed from the vessels immediately the cannulæ were tied *in situ*. The recipient's right femoral vessels were tied proximally and clamped distally, the cannulæ being inserted distally. The cannulæ of the recipient's artery and vein were also filled with citrate solution, and the rubber tubes leading from the donor's cannulæ were fitted to the recipient's cannulæ, artery to artery, vein to vein. The clamps were then removed from the recipient's vessels and then from the rubber tubes. Cross-circulation was thus established. As soon as this had definitely occurred, the rubber connecting tubes were clamped, while the loosely ligated vessels of the recipient, previously described, were tied off. The active circulation of the blood in the limb was tested by injecting saline into the rubber connecting tubes and watching its passage.

At the conclusion of the experiment the recipient's hindquarters were cut off at the level of the fourth lumbar interspace, the tail discarded, the part then bisected symmetrically and the two halves weighed.

Results

In the six cross-circulation experiments recorded, a variable period of time (18–152 min.) was allowed to elapse before the trauma was applied. This permitted any incompatibility which might exist to become evident. Isoagglutination phenomena appear to be unknown, or at most weak, in the cat [Wiener, 1934]. In some of the experiments the recipient's blood pressure was definitely lower than that of the donor prior to the establishment of the cross-circulation. This difference was in part attributable to the fact that the preliminary operative procedures on the recipient were more extensive and commenced earlier than those

TABLE I. Cross-circulation experiments: blood-pressure data at varying intervals before and after trauma (mm. Hg)

Exp.	Animal	Cat no.	B.P. prior to establishment of cross-circulation, but after operative manipulations	Cross-circulation established for x min. prior to trauma Value of x	B.P. prior to trauma	B.P. at 30 min. intervals after trauma						Survival time min.	Remarks
						30	60	90	120	150	180		
1	Recipient	58	150	100	102	86	—	—	—	—	—	Destroyed 25	—
	Donor	59	152		106	—	—	—	—	—	—		—
2	Recipient	60	106	18	90	96	—	—	—	—	—	Destroyed 28	Presumed increase in weight of traumatized hind-quarter, 16·5 g.
	Donor	61	134		156	88 (20 min.)	—	—	—	—	—		
3	Recipient	62	92	108	92	96	94	—	—	—	—	Destroyed 38	Presumed increase in weight of traumatized hind-quarter, 17·5 g.
	Donor	63	120		126	80	—	—	—	—	—		
4	Recipient	73	54	152	128	130	104	104	102	—	—	Destroyed 75	—
	Donor	74	164		106	80	56	—	—	—	—		—
5	Recipient	75	120	96	142	148	136	79 min. after original trauma intact foreleg hammered				42 min. after 2nd period of trauma	Presumed increase in weight of traumatized hind-quarter, 16·0 g.
	Donor	77	108		88	—	—	—	—	—	—	27	
6	Recipient	80	175	42	181	182	175	180	175	175	162	202	Presumed increase in weight of traumatized hind-quarter, 25 g.
	Donor	82	132		78	33	—	—	—	—	—	37	

applied to the donor. The blood pressure of the donor on the establishment of the cross-circulation fell in four out of the six experiments. This fall was probably mainly due to bleeding into the recipient's general circulation before the clamps could be applied to the connecting rubber tubes and the necessary ligatures applied. In order to ensure that the intubation technique of cross-circulation did not of itself contribute to the depression of blood pressure, which was sometimes evident on the establishment of cross-circulation, in each of two animals the femoral arteries and femoral veins were linked up so that the femoral artery of the right limb perfused the femoral vessels of the left limb. No obvious disturbance of blood pressure resulted.

Fig. 1. Exp. 3.

Despite the precautions taken to prevent bleeding from donor to recipient, there was frequently an apparent loss of blood from donor to recipient indicated by contemporaneous and opposite changes in the respective blood pressures on the establishment of cross-circulation. Following the first few minutes after the establishment of cross-circulation, the blood pressure of the donor generally remained stable, indicating that the depressant cause was no longer operative or was having a constant effect.

In five out of the six experiments the trauma inflicted caused fracture of one or more of the long bones, but in Exp. 6 care was taken not to break or injure bone. In this group of experiments death of the donor occurred within 25–75 min. from the time of application of severe trauma. This observation is contrary to the recent observations of Fender & Guptill [1936] on dogs. They anastomosed the carotid artery of the donor with the femoral artery of the recipient and the

femoral vein of the recipient with the jugular of the donor. The branches of the femoral artery and tributaries of the femoral vein were ligated to avoid a vascular shunt. Prior to cross-circulation the vascular bed of the recipient's limb was washed out with saline. Trauma was then applied to the transfused limb. The blood pressures of the donors showed a slight decline during and shortly after trauma, possibly due to the loss of blood into the vascular bed and tissues of the leg of the recipient. Thereafter the blood pressures returned to relatively high levels, and were maintained for 2 hr., when the experiments were concluded. Unfortunately no record of the recipient's blood pressures appears to have been taken.

As it was considered that little information could be derived from the experiments following the death of the respective donors—the injured limbs of the recipients being no longer transfused with blood—in five out of the six experiments the recipients were killed off, at times varying from a few minutes up to slightly over an hour after the donor's death. In Exp. 6, however, a record of the recipient's blood pressure was taken up to the point of death which occurred 3 hr. 22 min. after the trauma. The animal in question had been on the operating table for 10½ hr. The apparent cause of death was œdema of the lungs.

In the fifth experiment of this series, the donor died 27 min. after the application of trauma, and 52 min. later one of the intact forelimbs of the recipient was traumatized. Shortly afterwards the other forelimb was similarly hammered. The recipient died 42 min. after this period of trauma.

Fat embolism might have been considered as a cause of death in these experiments, had not the donor in Exp. 6 also died. In this experiment the bones were not injured. A contributory cause of the fall in blood pressure is the loss of fluid into the injured limb. This loss presumably occurs slowly and an attempt to compensate for it would be the natural reaction of the organism. The resultant fall in blood pressure due to this cause alone would probably therefore be slight and would only account for a fraction of the general fall in pressure. The maximum observed gain in weight of an injured limb was 25 g. This gain in weight was equivalent to a loss of approximately 11 p.c. of the original blood volume. Such a loss, although slight, is of more serious consequence than at first appears evident, for it occurs during a period when the donor's blood pressure is, largely as the result of the cross-circulation procedures, definitely below its normal value.

Observations on the effect of hæmorrhage indicated that, in the case of an animal with a normal blood pressure, the removal of blood to the extent of 11 p.c. of the blood volume caused only a slight depression in

blood pressure. This was done on an animal in which cross-circulation was effected between its right and left hindlimbs. Withdrawal of blood to the extent of 15–20 p.c. in a similar experiment caused a marked depression in blood pressure which was not fatal within the time of observation. On the other hand, the removal of 35 ml. blood, corresponding to 8·5 p.c. of the blood volume of the donor of a cross-circulation experiment produced a fatal result within 44 min. from the commencement of the withdrawal of blood. Removal of a corresponding amount of blood from the recipient caused some depression in blood pressure but was not fatal. The blood pressures of both animals were 120 mm. Hg. In this experiment the bloods were removed from the external jugulars.

Several attempts were made to demonstrate the presence of a depressor substance in the blood removed from the injured limb of an animal during shock. Blood was generally collected from the femoral vein of the injured side and either reinjected into the same animal or into a normal animal. In neither instance was there any evidence of anything but a very slight initial depression of blood pressure on injection. A depressor substance with a definite but transient effect developed in normal citrated blood on standing. This is probably due to an increase in the adenosine equivalent [Barsoum & Gaddum, 1935].

Undoubtedly the local loss of fluid into the tissues adjacent to the site of injury is an important factor but, in addition, there is evidence of a more generalized passage of fluid out of the blood stream. Transfusion of gum arabic saline during the onset of the fall in blood pressure causes only a very temporary restoration in the blood pressure. The accumulation of fluid in the damaged limb did not account for all the fluid "lost" from the circulation. There appears to be a critical level of blood pressure in the region of 80–90 mm. which the organism tries to maintain. Death rapidly ensues if the pressure falls below this level. The same thing is observed in cases of clinical shock.

The evidence appears to weigh in favour of a loss of blood volume as the major factor in the production of the low blood pressure in shock. In support of this we have observed that when trauma was applied to a limb around which a tourniquet had been so placed that the femoral artery was the sole communication between the limb and the trunk, the animal died within 48 min. The increase in weight of the traumatized extremity was 31 g., representing about 21 p.c. of the blood volume.

The cause of the relatively local loss of blood volume may conceivably be due to the action of a local humoral mechanism. Simonart [1930] concluded that the presence of an intact nerve supply was an essential

feature in the production of traumatic shock, and the observations of O'Shaughnessy & Slome [1934–5] on the effect of nerve section and of spinal anæsthesia indicate that with these measures shock either failed to develop or was delayed. We have confirmed these observations on the effect of nerve section in a series of cats receiving chloralose and also chlorazol fast pink. In order to be certain that all the nerves were divided the hindlimb was disarticulated at the hip joint, leaving only the iliac vessels intact. The vascular sheaths were also cut across. These vessels were, therefore, the only connexion between the body and the limb. In these experiments it was found that, after the application of severe trauma to the limb, the onset of shock was delayed and there was no appreciable fall of blood pressure until a period of an hour had elapsed. Attempts to weigh the disarticulated limbs from a spring balance before and after trauma were unsatisfactory. These experiments were controlled by observations in another group of similar experiments in which the main nerves to the limb as well as the iliac vessels were left intact. In this latter series severe trauma to the limb was followed in a very short time by a marked fall in blood pressure. These experiments are undoubtedly of a crude nature, for the disarticulation of itself would have been sufficient to produce some shock during the period of record. The difference noted in the behaviour of these two series of animals suggests that an intact nerve supply is one of the essential factors in the induction of shock. It is suggested that probably there is the production of some substance in the injured area which does not circulate in the blood, but which causes directly or indirectly a loss of blood volume, and that the induction of this state is dependent on the integrity of the nervous paths to the injured zone.

In none of the experiments was there any evidence that traumatization of the recipient's transfused limb caused anything but a temporary disturbance of the recipient's blood pressure. This is directly opposed to the finding of O'Shaughnessy & Slome. In their two recorded cross-circulation experiments [1934–5, 1935] the recipients went into shock and died, and in one they found that trauma caused death of the donor some 2 hr. after the injury and actually before the recipient went into shock. They offer no explanation to account for the donor's death in this experiment, attributing death of the recipient to a centripetal nervous discharge from the injured area. It may be that under nembutal anæsthesia such as was used in our experiments, reflexes are relatively unimportant. In their experiments O'Shaughnessy & Slome used chloralose, and therein may rest the explanation for this discrepancy.

SUMMARY

1. Severe trauma applied to the thigh muscles of anæsthetized cats caused a marked fall in blood pressure, followed by death within 100 min.

2. By means of cross-circulation experiments it was found that trauma applied to a transfused hindlimb of the recipient animal caused a marked fall in the blood pressure of the donor, followed by death within 75 min. The blood pressure of the recipient was practically unaffected by the injury.

3. Local fluid loss as determined by comparative weighings of the transected hindquarters is a contributory cause, if not the main cause of this marked fall in blood pressure. The induction of this state appears to be dependent on the integrity of the nervous paths to the injured zone. There is some evidence that, in addition to the local fluid loss, the blood volume may be reduced by a more generalized "loss".

In conclusion we wish to express our thanks to Prof. E. P. Cathcart, Prof. G. M. Wishart and Prof. D. M. Blair for their helpful criticism.

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QUANTITATIVE DATA ON THE INHIBITION OF ŒSTRUS BY TESTOSTERONE, PROGESTERONE, AND CERTAIN OTHER COMPOUNDS

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The inhibition of recurrent œstrous cycles in the lower rodents and in other species during the period of activity of the corpus luteum has been extensively investigated and has given rise to much controversy. The earlier work is reviewed by Parkes [1929], and later data are discussed by de Fremery *et al.* [1934] and Selye *et al.* [1936*a*]. There is no doubt that œstrus remains in abeyance in a large number of animals during the period of luteal function, but there is little evidence to show that this is actually due to the luteal hormone progesterone, and any positive effects produced experimentally have involved the use of large doses of the hormone. Thus Selye *et al.* [1936*b*] inhibited œstrus in rats by the daily injections of 4·0 mg. of crystalline progesterone, while Phillips [1937] obtained a similar result with daily injections of 1·5 mg. De Fremery *et al.* inhibited the appearance of cornification in ovariectomized mice receiving a little over a unit of œstrin by mouth by the injection of 3 rabbit units of progesterone. Similar results were obtained by Allen & Meyer [1935] with luteal extracts. The doses used in these different experiments do not bear accurate comparison owing to differences in the method of standardization. The international unit of progesterone, established in 1935, is 1 mg. of the crystalline hormone.

The specificity of the action of the luteal hormone described above has been called in question owing to the contradictory nature of the evidence, the large doses of hormone required in the positive experiments, and the fact that inhibition of œstrus has been obtained with other substances (e.g. adrenaline, Robson, 1932). Since a number of pure synthetic compounds with "male hormone" properties has recently been prepared,

it became desirable to investigate their action on the sex cycle, especially as accurate quantitative work is now possible. It was soon found that comparatively small doses of the "male hormone" testosterone were able to inhibit œstrus and the action of œstrin in the mouse [Robson, 1936]. Further quantitative investigations were then undertaken with a number of other compounds and the doses of crystalline progesterone necessary to bring about these effects in mice were also determined. This was followed by an attempt to elucidate the mechanism by which these substances inhibit the œstrous cycle. The investigation will be described in these consecutive steps.

General methods

All the experiments were performed with mice kept under constant conditions on a diet of oats, rye bread, milk, water and lettuce. Not more than five animals were kept in each of the metal cages.

Ovariectomy was performed by the dorsal route and the animals were not used for at least a fortnight after the operation.

I am greatly indebted to Dr Miescher of Ciba, Ltd. for the supply of the various compounds with male hormone properties; to Messrs Schering, Ltd., for the supply of progesterone in oil (proluton) and to Prof. Laqueur for a supply of crystalline progesterone; and to Dr Macbeth of Organon Ltd. for the supply of œstradiol. The crystalline substances were dissolved in benzene; the requisite amount of oil of sesame was then added and the benzene blown off.

The gonadotropic hormone preparation from human pregnancy urine (M2) was stored as a dried powder and solutions in saline were made up every 4 days and stored in the ice chest. The rabbit ovulating unit of this preparation was about 0·5 mg.

Results

I. *Inhibition of activity of the natural ovarian secretions. Experiments on intact animals*

Daily vaginal smears were taken from a number of mice for 4 weeks at least, and only those were used which showed regular œstrous cycles. The substances were injected twice daily in 0·1 or 0·05 c.c. of oil, with the exception of testosterone propionate which was administered only once a day in a similar amount of oil. Injections were spread over periods of 11–21 days. Control injections of oil had no effect on the œstrous cycle. The results are shown in Table I.

TABLE I. The effects on the œstrous cycle of testosterone and testosterone propionate

Substance	Daily dose in μg.	No. of animals	Effect on œstrous cycle
Testosterone	200	5	Inhibition
	100	1	,,
	40	1	,,
	20	1	,,
Testosterone propionate	10	2	Partial inhibition
Progesterone	400	1	Inhibition
	200	1	,,
	100	2	No inhibition

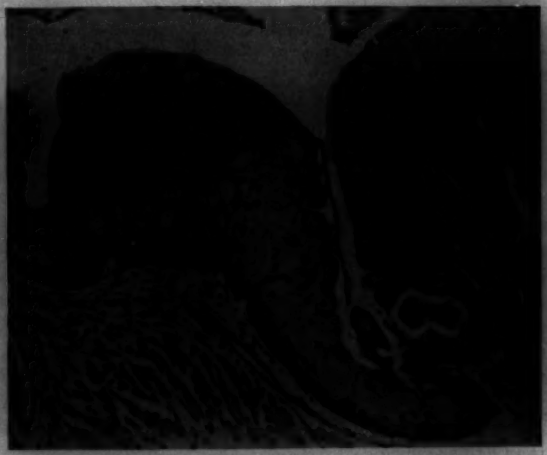

Fig. 1. The vaginal mucification in a mouse in which œstrus was inhibited by testosterone injections. ×175.

It will be seen that testosterone is 5–10 times as active as progesterone in inhibiting œstrus. The effect appeared rapidly and no vaginal cornification was observed while testosterone was given in daily doses of 20μg. or more. The daily injection of 10μg. of testosterone propionate also produced inhibitory effects, but did not completely inhibit the œstrous cycles. After cessation of the injections normal cycles reappeared within comparatively short periods. Two animals were kept with males during the period when testosterone was being injected but no vaginal plugs were found. One animal mated at the first œstrus following cessation of the injections and became pregnant.

Two animals received 200 μg. of testosterone per day and were killed immediately after the period of injections. In both cases the vagina showed a marked degree of mucification. This is illustrated in Fig. 1.

Similar experiments with progesterone showed that the daily administration of 400 μg. or 200 μg., in two doses, completely inhibited œstrus, but the injection of 100 μg. per day produced little or no effect on the cycles.

A number of other substances was then given with the following results: androsterone completely inhibited œstrus when the daily dose was 200 μg.; androstanediol when the daily dose was 100 μg. and androstenedione when the daily dose was 400 μg. Partial inhibitory effects were obtained with smaller doses. Hence the potency of progesterone, androsterone, androstanediol and androstenedione in the inhibition of œstrus was approximately of the same order. On the other hand, daily administration of 400 μg. of androstenediol produced no inhibitory effects.

II. *Inhibition of activity of injected œstrogens. Experiments on spayed animals*

As it appeared possible that the substances tested might produce their effects by directly antagonizing the action of œstrin on the vagina, a number of experiments was performed in which the substance was injected together with œstrin into spayed mice. The changes in the experimental animals were compared with those in the control groups of animals receiving only the œstrogenic compounds. The doses of œstrin were so adjusted as to produce full cornification in over 50 p.c. of a group of animals. Thus œstrone was injected in total doses of 0·1 or 0·2 μg., as it has been previously shown [Hain & Robson, 1936] that such doses give a positive effect in more than half the animals of a group of twenty. The dose of œstradiol was 0·05 μg., and this also produced full cornification in more than half the mice.

The dose of œstrin was dissolved in 0·2 or 0·4 c.c. of oil of sesame together with the compound whose inhibitory action was being tested. Each animal received four injections (each of 0·1 or 0·05 c.c.) on the morning and evening of two consecutive days, and vaginal smears were subsequently taken five times, once on the evening of the day following the last injection, three times (morning, afternoon and evening) on the next day and once on the morning after that. An attempt was made to read the smears in a roughly quantitative manner, recording the various cells present, thus allowing of an accurate comparison between the effects produced in various groups of animals. The majority of experiments were performed on groups of twenty mice, but ten mice were occasionally used when pilot experiments to determine approximately the effective dose

of a certain compound were made. In a majority of cases a control experiment with œstrin only was performed. The results are collected in Table II.

TABLE II. The effect of addition of androgens or progesterone on the vaginal response of ovariectomized mice to œstrone or œstradiol

Substance	Dose μg.	Dose of œstrone μg.	Ratio substance: œstrone	Inhibitory effect on vaginal response to œstrone
Testosterone	400	0·1	4000 : 1	+ + + +
	800	0·2	4000 : 1	+ + + +
	100	0·2	500 : 1	+ + +
	50	0·2	250 : 1	+
	100	1·0	100 : 1	Nil
Testosterone propionate	500	0·2	2500 : 1	+ + +
	50	0·2	250 : 1	+ +
	20	0·2	100 : 1	Nil
	500	1·0	500 : 1	+ +
Progesterone	750	0·2	3750 : 1	+ +
	500	0·2	2500 : 1	Nil
	200	0·2	1000 : 1	Nil
	100	0·2	500 : 1	Nil
Androsterone	800	0·1	8000 : 1	Nil
	1000	0·2	5000 : 1	Nil
	500	0·2	2500 : 1	Nil
Androstanediol	1000	0·2	5000 : 1	+ +
	500	0·2	2500 : 1	Nil
Androstenedione	1000	0·2	5000 : 1	+ +
	500	0·2	2500 : 1	Nil
	200	0·2	1000 : 1	Nil
Androstenediol	1000	0·2	5000 : 1	Nil
	500	0·2	2500 : 1	Nil
Trans-dehydro-androsterone	1000	0·2	5000 : 1	Augmentation
	400	0·1	4000 : 1	Slight augmentation

Substance	Dose μg.	Dose of œstradiol μg.	Ratio substance: œstradiol	Inhibitory effect on vaginal response
Testosterone	100	0·05	2000 : 1	+ + +
	25	0·05	500 : 1	+ + +
	15	0·05	300 : 1	+ +
	100	0·25	400 : 1	+ +
Testosterone propionate	500	0·20	2500 : 1	+ + +
	250	0·05	5000 : 1	+ + + +
	7·5	0·05	150 : 1	Nil
Progesterone	250	0·05	5000 : 1	Nil
	750	0·05	15,000 : 1	+ + +
Androsterone	1000	0·05	20,000 : 1	Nil
Androstanediol	250	0·05	5000 : 1	+ +
Androstenedione	250	0·05	5000 : 1	Nil

It was found that the administration of 400μg. of testosterone together with 0·1μg. of œstrone completely inhibited any effects on the vagina that could be detected by smearing. Thus the smears of animals injected with such a mixture were similar to those of uninjected spayed

animals. As the dose of testosterone was decreased the inhibitory action became less. None of the animals receiving 100 μg. of testosterone together with 0·2 μg. of œstrone showed full cornification, though partial effects were obtained in some. But when the dose of the testosterone, given with 0·2 μg. of œstrone, was decreased to 50 μg. full cornification was found in 20 p.c. of the animals, as compared with 60 p.c. in a group receiving 0·2 μg. of œstrone only.

The injection of a single dose of testosterone propionate given at the same time as the first dose of œstrone was also effective in the inhibition of cornification, even when only 50 μg. of the compound were given.

When a dose of œstrone of 1·0 μg. was given together with 100 μg. of testosterone, all the mice showed complete cornification, so that no inhibitory effects could be demonstrated under those conditions.

Similar experiments were then performed, using progesterone instead of testosterone, and it was found that much larger doses of the luteal hormone had to be used to produce any inhibitory effect. Thus no inhibition of vaginal cornification was obtained when 500 μg. of progesterone were given together with 0·2 μg. of œstrone, and it was only when the dose of the luteal hormone was raised to 750 μg. that some inhibitory effect was obtained, cornification being produced in only 10 p.c. of the group of animals. Hence testosterone is about fifteen times as effective as progesterone in the inhibition of the vaginal effects of œstrone.

The action of a number of other substances was then investigated. It was found that androstanediol and androstenedione exerted inhibitory effects in doses similar to that of progesterone: 1000 μg. of either of these substances given together with 0·2 μg. of œstrone caused a definite decrease in the vaginal cornification reaction. On the other hand, androsterone and androstenediol were without any such actions in doses up to 1000 μg.

Trans-dehydro-androsterone had an effect opposite to that of testosterone and of progesterone. When 1000 μg. of this substance were given together with 0·2 μg. of œstrone full cornification was produced in all the animals injected. Since this substance is known to possess œstrogenic activity [Butenandt & Kudszus, 1935; Deanesly & Parkes, 1936] this result is not surprising.

There is evidence that the œstrogenic hormone actually secreted by the ovary is œstradiol [MacCorquodale *et al.* 1936], and it was therefore decided to investigate the relations between this substance and the various compounds mentioned above, in their effects on the vagina. The experiments were performed in exactly the same way as those with

œstrone. The total dose of œstradiol given in all cases (except one) was 0·05 μg., which dose was at least as potent in producing vaginal cornification as 0·2 μg. of œstrone.

It was found that the action of œstradiol was inhibited by smaller doses of testosterone than were necessary to inhibit that of œstrone. Thus no full cornification was produced in any of the mice injected with 0·05 μg. of œstradiol and 15 μg. of testosterone. To produce a similar inhibition of the action of an equivalent amount of œstrone, i.e. 0·2 μg., a dose of testosterone of more than 50 μg. was necessary so that testosterone inhibited the action of œstradiol more than 3 times as effectively as that

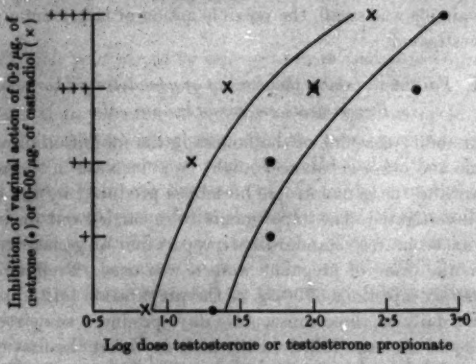

Fig. 2.

of œstrone. The inhibition of the cornifying actions of œstrone and of œstradiol by various doses of testosterone (or testosterone propionate) is graphically represented in Fig. 2, which shows that the vaginal activity of œstradiol is inhibited by smaller doses of testosterone (or its propionate) than the vaginal action of œstrone. Similar differences were not found with progesterone. When 250 μg. of the luteal hormone were injected together with 0·05 μg. of œstradiol no inhibition of the vaginal cornification reaction was produced. Androstenedione, too, did not prove to be more effective in the inhibition of the action of œstradiol than in the inhibition of that of œstrone, since 250 μg. did not produce any inhibition of the action of 0·05 μg. œstradiol. On the other hand the administration of 250 μg. of androstanediol together with 0·05 μg. of œstradiol did not produce full cornification in any of the animals.

Androsterone in doses of 1000 μg. did not in any way interfere with the action of œstradiol. It was thought possible that cholesterol might have such an action, but 2·0 mg. of this substance had no inhibitory effect.

In view of the well-known relationship between the adrenal cortex and the sexual activity it was possible that the compound with male hormone properties recently isolated by Reichstein [1936] from the cortex might have an action similar to that of testosterone. 100 μg. adrenosterone (for the supply of which I should like to thank Prof. Reichstein) were administered together with 0·05 μg. of œstradiol, but the vaginal response remained unimpaired. As the quantity of adrenosterone available was small, the possible action of larger amounts could not be investigated.

III. *Failure to inhibit the activity of gonadotropic hormone.*

Experiments on immature animals

The possibility that one of the factors in the inhibition of œstrus by testosterone and other substances might be a decrease in the reactivity of the ovaries to the gonadotropic hormones produced by the pituitary was next investigated. The experiments were carried out on immature mice and an accurately standardized preparation of gonadotropic hormone from the urine of pregnant women was used. Preliminary tests showed that six injections of 50 μg. of the preparation (M2, dissolved in saline) twice daily on three consecutive days produced some response in the majority of the mice killed on the fifth day after the first injection, and all the animals used in the experiments described below received such injections. Forty-five immature mice were used in the experiment. Sixteen of these were injected with testosterone propionate and gonadotropic hormone, fourteen mice received progesterone and gonadotropic hormone, while the control group of fifteen mice received only gonadotropic hormone. Ten of the first group of animals received 500 μg. of testosterone propionate in oil together with the first injection of gonadotropic hormone, and the other six mice received a total of 1000 μg. of testosterone propionate in oil. The first dose of 250 μg. was given on the day before the injections of M2 started, and three doses of 250 μg. each were given on the 3 days on which gonadotropic hormone was injected. Five of the mice in the second group received a total of 2000 μg. of progesterone, while the other nine received 1000 μg. The first dose was given on the day before the injections of M2 started and the other three doses were injected on the next 3 days.

The results of the effects on the ovaries in the three groups of mice are shown in Table III. It will be seen that there is no appreciable difference. These experiments therefore suggest that neither testosterone nor progesterone inhibits the response of the ovary of the mouse to gonadotropic hormone.

TABLE III. Absence of effect of testosterone and progesterone on the response of the ovaries of immature mice to gonadotropic hormone. See text for details of injections

Group	No. of animals	Injections in addition to gonadotropic hormone	No. of animals showing: No effect on ovaries	Follicular growth	Blood spots
A	16	Testosterone	4	10	8
B	14	Progesterone	3	9	5
C	15	None	3	10	8

IV. *Possible part played by androgens in maintaining pregnancy. Experiments on spayed pregnant animals*

The fact that testosterone exerts on the œstrous cycle an action similar to that of progesterone suggested the possibility that it might be effective in the maintenance of pregnancy. The finding that testosterone inhibits the reactivity to oxytocin of the rabbit's uterus [Robson, 1937] and produces progestational proliferation of the endometrium [Klein & Parkes, 1936] was also in favour of such a possibility.

Since there are, however, no data as to the amounts of progesterone necessary to maintain pregnancy in the mouse, this question was first investigated.

The hormone was injected daily in solution in oil, the first dose being given one or two days before the removal of the ovaries on the fourteenth or fifteenth day of gestation. It was found that the daily administration of 400 μg. of progesterone was not sufficient to maintain pregnancy, abortion following 24–48 hr. after the operation. On the other hand, gestation was maintained in all animals receiving 1000 μg. of progesterone per day.

Mice were similarly injected with 1000 or 2000 μg. of testosterone propionate a day, the first dose being given 2 days before the operation, but removal of the ovaries produced abortion in all cases. Androstanediol, too, was not capable of maintaining pregnancy in the ovariectomized mice.

In a number of experiments mice were ovariectomized during pregnancy and injected with both progesterone and testosterone or androstanediol. It was found that the dose of the luteal hormone effective in the maintenance of pregnancy was somewhat less when testosterone or androstanediol was injected at the same time.

DISCUSSION

These experiments show that both testosterone and progesterone can inhibit the normal œstrous cycles in mice. In both cases a mucified condition of the vagina results, and no mating occurs during the period of injections. The doses of testosterone necessary to produce this inhibition

are much smaller than the quantities of progesterone. It is possible that differences in the rate of absorption of these hormones may account to some extent for the differences in their effects observed in the present experiments. Indeed, there is reason for believing that the amounts of these hormones necessary to produce certain effects when given by injection may be greatly above the amounts produced under physiological conditions, since much smaller quantities have produced effects under different experimental conditions, e.g. by the implantation of crystals of the hormone [Deanesly & Parkes, 1937]. At the same time the possibility remains that the so-called male hormone may play a part in controlling the sex cycle, and the recent extraction from the ovary of a substance possessing male hormone properties [Parkes, 1937] lends support to such a supposition.

The results of the direct interaction between œstrin and testosterone (or progesterone), as investigated in ovariectomized animals, suggest that an action on the end organ (i.e. the vagina) may play some part in the mechanism of the inhibition of œstrus. A quantitative examination of the data shows, however, that this direct interaction cannot be the only factor involved. It has been found that the daily administration of 20 μg. of testosterone may produce inhibition of œstrus within some 5 days of the injection of the first dose. Hence, assuming that all this hormone is stored and effective, as small a total dose as 100 μg. inhibits the appearance of the next œstrus. Now such a dose will prevent the full action of five units of œstradiol (0·25 μg.) in only 50 p.c. of animals and will not prevent the appearance of cornification in any animals when injected together with 10 units of œstrone. Since there is good evidence that the doses of œstrin produced during normal œstrus are of the order of 200 units or more [Marrian & Parkes, 1930] it can be concluded that the inhibitory action of testosterone on the œstrous cycle is not merely due to a direct antagonism of the action of œstrin on the vagina. Indeed, the results suggest that the production of the œstrous hormone is actually interfered with. Since the evidence does not favour the possibility that ovarian activity in response to gonadotropic hormone stimulation is prevented by testosterone, it seems possible that the testosterone inhibits the secretory activity of the pituitary and that this is one of the important factors in the inhibition of œstrus. Such a point of view is, of course, in accordance with the hypothesis and extensive experimental data already put forward by Moore & Price [1932]. The possibility that the mechanism of œstrus inhibition involves an alteration in the rate of excretion of œstrin has, however, also to be considered.

The substances which have been investigated for their effect on the reaction of the vagina of ovariectomized animals to œstrin can be divided into four groups: viz. (1) testosterone and androstanediol, which inhibit the action of œstrin, the dose necessary to inhibit the biological unit of œstradiol being smaller than the dose needed to inhibit the unit of œstrone. It must, however, be noted that the ratio between the amounts of testosterone necessary to antagonize a given weight of oestrone and œstradiol respectively seems to be very similar and to be about 250 : 1. The finding that the ratio between the amounts of œstrone or œstradiol and testosterone is the important factor on determining the inhibitory

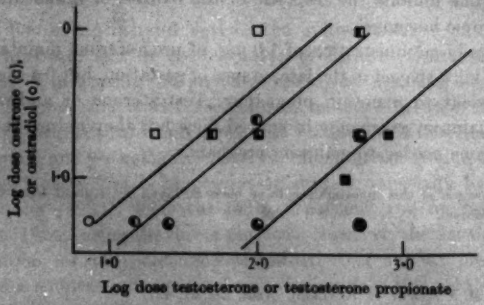

Fig. 3. Inhibition: □ ○ = nil; ◧ ◐ = + + ; ■ ● = + + + ; ■ ● = + + + + .

action produced is graphically illustrated in Fig. 3. With androstanediol this ratio is about 5000 : 1. Much smaller ratios have been found for the antagonistic action of these hormones on the prostate, viz. 7 : 1 for testosterone propionate and œstrone and 50 : 1 for androstanediol and œstrone [Zuckerman & Parkes, 1936; Zuckerman, 1936]. (2) Progesterone and androstenedione inhibit the action of œstrone, but when given with œstradiol in a similar ratio (5000 : 1) there is no inhibition of the vaginal effect. (3) Androsterone and androstenediol which do not, in the doses used, antagonize the action of œstrone or, in the case of androsterone, of œstradiol. (4) *Trans*-dehydro-androsterone which produces an augmentation of the vaginal effect when injected together with œstrone.

Further investigations are necessary before the actions of testosterone during pregnancy can be adequately discussed.

Summary

The œstrous cycle in mice can be inhibited by the daily administration of 20 μg. of testosterone or 200 μg. of progesterone. The effect of a number of other substances is described.

The vaginal cornifying action produced by the injection of œstradiol or œstrone into spayed mice can be inhibited by the simultaneous administration of testosterone or progesterone (or certain other substances). The doses of testosterone necessary to produce such inhibition are much smaller than those of progesterone.

No evidence could be obtained to show that either testosterone or progesterone inhibits the response of the ovaries of immature mice to gonadotropic hormone.

The daily administration of 1·0 mg. of progesterone maintains pregnancy in mice spayed in the later stages of gestation, but 0·4 mg. daily is not sufficient to maintain pregnancy. Testosterone or androstanediol cannot maintain pregnancy in spayed mice but they reduce the dose of progesterone needed to maintain pregnancy.

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SOME FACTORS INFLUENCING VENOUS PRESSURE IN MAN

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In 1906 Recklinghausen devised the indirect method of estimating venous pressure, and in 1910 Moritz & Tabora demonstrated the feasibility of measuring the pressure directly by inserting a needle into a vein and connecting it with a water manometer. Since then the relative ease of these procedures has given rise to numerous investigations on both normal and pathological material. Much of the ground covered in the present paper has been previously studied, especially by Carrier & Rehberg [1923], using the indirect method, and by Bedford & Wright [1924], using the direct method. However, the development of a technique for the continuous registration of venous pressure has provided a suitable inducement for further work in this field.

The basic concept underlying the present investigation may be stated as follows. The pressure in a flowing system is dependent on the input pressure, the resistance offered to the flow and the back pressure, if any. The veins of man form such a system in which the input pressure is derived from the blood pressure within the capillaries. Some of the variable factors opposing the flow are the hydrostatic pressure of the column of fluid and the viscous resistance of the venous channels. In such a system, variation of either of these two elements or of the input pressure should cause a predictable result on the pressure measured at any point. The results of variation in some of these factors on venous pressure in man are reported in this paper, together with the deductions arising therefrom.

Method

Venous pressures have been recorded by an apparatus consisting of a glass T-tube manometer of 3 mm. bore, filled with a sterile solution of 1 p.c. sodium citrate in normal saline. One end of the horizontal section

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of the manometer was attached by a short piece of pressure tubing to a 16 gauge needle. This needle was inserted into a vein. Similar tubing connected the other end of the cross-piece to a reservoir containing the solution of citrate. During periods of recording a screw clip was used to separate the reservoir from the manometer, but it was frequently released to flush out the needle. This open system permitted direct readings of the pressure to be made. Continuous optical records were obtained by connecting the open end of the manometer by a closed-air system to a rubber diaphragm which supported a mirror. This mirror reflected a narrow beam of light on to a strip of moving bromide paper. Usually two diaphragms of different elasticity were used at the same time; in all experiments the more sensitive tambour was so calibrated that a deflexion of 0·5 cm. on the paper was approximately equivalent to a change in pressure of 1 mm.-H_2O. The recorded changes in pressure were only relative, as the direction in which the point of the needle was placed in the vein influenced the magnitude of the changes but not their direction. Except where otherwise stated, the needle was inserted with the point directed towards the heart.

Using a small plethysmograph similar to that described by Sewall & Sanford [1890] and by Goetz [1935], the volume of a digit was recorded simultaneously with the venous pressure. The method of recording, that of Bolton *et al.* [1936], consisted of connecting the plethysmograph by a closed-air system to a tambour which reflected a beam from a mirror attached to its outer surface. The respiratory phases were recorded by a similar capsule which was connected to a balloon strapped to the chest by an elastic bandage.

Six normal subjects have been used in this investigation. Venipuncture has been performed twelve times on the median basilic vein at the elbow and five times on the great saphenous vein at the ankle. The former vein drains both muscle and skin while the latter receives blood mainly from skin. In three further subjects, normal for the purpose of this investigation, the pressures in the internal jugular bulb have been recorded.

The experimental procedure consisted in the subject quietly standing, sitting, or lying. When seated or lying, the limb from which records of venous pressure were to be made was comfortably supported so that its position could be easily varied. The needle was then inserted, and its position in the vein was verified by applying a tourniquet proximal to the needle and observing if a prompt rise in pressure resulted. The variations in venous pressure associated with quiet breathing were

then recorded and at intervals the subject was requested to take a deep breath. Various stimuli, such as the application of a small piece of ice to one of the other limbs for a few seconds, were given from time to time in order to produce vasoconstriction of the peripheral blood vessels. The mechanism of the changes in venous pressure following such stimuli were investigated (1) by immersing an indifferent limb in water at 45 or 15° C., to bring about dilatation or constriction of the peripheral vessels, (2) by altering the position of the limb, and (3) by cutting off the circulation distal to the needle by a cuff inflated to the pressure of 250 mm. Hg. Other procedures will be described in the text.

Results

A. *Pressure*

The pressures in different veins under various conditions have been compared. In the supine posture with the arm abducted at right angles from the body and held below the level of the chest, the pressure recorded from the median basilic vein was approximately equal to the height of the manubrium sterni above the point of insertion of the needle. In the supine posture the pressure obtained from the long saphenous vein was again equal to the height of the manubrium sterni above the insertion of the needle. The pressure in the internal jugular bulb when the subject was supine and the head held in line with the trunk reached also to the level of the manubrium sterni. If the point of insertion of the needle was raised above the level of the manubrium sterni, as when the arm was elevated, the residual pressure, that is, the pressure remaining in the median basilic vein, varied in eight experiments between 1 and 3 cm. H_2O. The degree of elevation above the manubrium sterni did not appear to affect this residual pressure and at no time was a negative pressure observed. Carrier & Rehberg [1923] and Bedford & Wright [1924] have also observed positive pressures when the arm was elevated. The former authors found the residual pressure to be in the neighbourhood of 5 cm. H_2O and that the pressure did not change with successive elevations once a vein was above a level some 5–7 cm. below the clavicle. They point out, however, that their method was not absolutely accurate at these lower pressures and that direct observation showed the vein to collapse at a level just below the clavicle.

In the erect posture the pressure in the long saphenous vein at the ankle of three subjects was equal to the difference in level between the point of recording and the third interspace at the sternal border. This

finding agrees with that of Carrier & Rehberg [1923] but is less than that found by Smirk [1936]. In the same subjects, in the median basilic vein when the arm was dependent, the pressure was equal to the difference in level between the needle and the upper border of the sternal notch. Radiography showed the sternal notch to be slightly above the level of the first rib where it is crossed by the subclavian vein. If the arm was gradually raised the pressure in the median basilic vein progressively diminished until the arm was level with the first rib, at which point pressures of 1–3 cm. H_2O were observed. On raising the arm above this level no significant alteration occurred in this residual pressure. The pressure in the jugular bulb in the erect position varied between 0 and 0·5 cm. H_2O and here also a negative pressure was at no time observed.

These findings demonstrate the wide difference in venous pressures existing in different veins in the upright position and also the variations in pressure in the same vein when the position of the body or the position of the limb is altered. Furthermore, these findings show that the pressure in the veins is capable of lifting the blood to an adequate level for its return to the heart without the assistance of the pumping action of the muscles.

B. *Alteration in the state of the peripheral vessels*

(*a*) *Vasodilatation.* The effect of cutaneous vasodilatation on the pressure in the median basilic vein was observed on five occasions. This was carried out in a subject lying horizontal with the arm, from which the venous pressure was being registered, supported below the level of the manubrium sterni and abducted at an angle of 90° from the body. The subject lay exposed to the cold atmosphere of the room and in this state the finger temperature was low, indicating a state of peripheral vasoconstriction. One leg was then immersed and kept gently moving in a tank of water at 44–45° C. As a result peripheral vasodilatation took place in the finger vessels of the arm under investigation. No change in venous pressure was recorded during the dilatation of the peripheral vascular bed. That dilatation of the peripheral vessels occurred was shown by the rise in temperature of the fingers of the same hand and by the increase in finger volume and the increase in the pulse amplitude as recorded by a plethysmograph from the digit of the other hand.

(*b*) *Transient vasoconstriction.* The effect of transient vasoconstriction of the peripheral vessels on venous pressure has been observed fifty times in six individuals. Such vasoconstriction is known to occur as an active response to painful and cold stimuli given to subjects whose

peripheral vessels are in a dilated state [Stürup *et al.* 1935]. When the subject is cold and the peripheral vessels are already constricted, no further constriction is brought about by such stimuli. It was found in

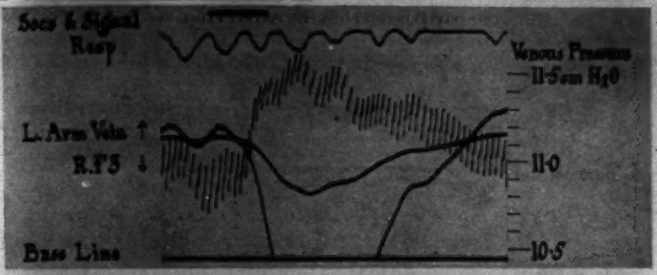

Fig. 1. Record from basilic vein of left arm and of volume of right middle finger (R.F. 3). Subject warm and peripheral vessels dilated. Arm below chest level. Arrows indicate direction of increase in pressure or of volume: in respiratory record downward stroke is inspiratory phase: these are the same for all records. The appended scale in this and the following figures refers to the venous tracing with the greatest excursion and shows approximately the magnitude of pressure change. Line marked "Base Line" in this and following records is obtained from a mirror attached to a stand holding tambours. Ice to right thigh at signal.

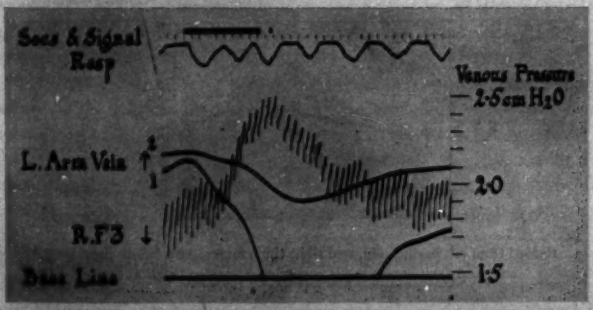

Fig. 2. Record from left basilic vein and right third finger. Subject warm and peripheral vessels dilated. Arm above level of chest. Ice to right thigh at signal.

these six subjects when their cutaneous blood vessels were in a state of dilatation that ice applied to the skin of a part of the body for a few seconds produced a transient fall in venous pressure; this fall was

observed in the median basilic and long saphenous veins. The fall in venous pressure was synchronous with the diminution in digit volume and returned to the original level as the diminution in finger volume disappeared (Fig. 1). These changes in venous pressure were also observed when the limb was above the level of the manubrium sterni (Fig. 2). The

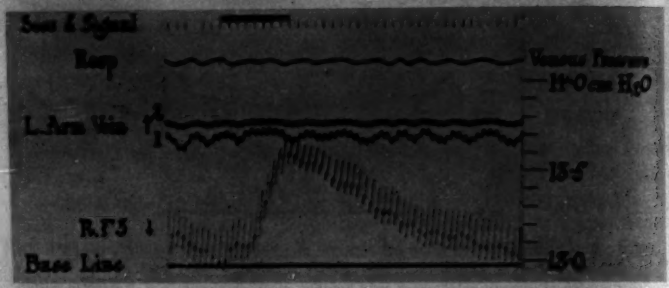

Fig. 3. Record from left basilic vein, and right third finger. Subject warm and peripheral vessels dilated. Arm below level of chest and peripheral circulation occluded. Ice to thigh at signal.

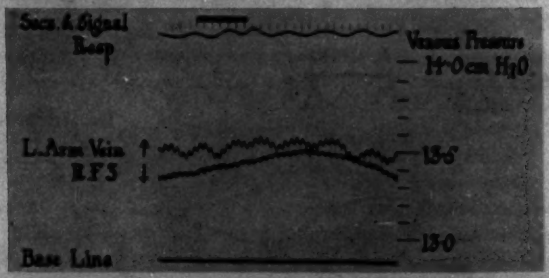

Fig. 4. Record from left basilic vein, and right third finger. Arm below chest level. Subject cold and peripheral vessels constricted. Ice to thigh at signal.

fall in venous pressure, however, did not occur if the circulation distal to the needle had been occluded by inflating a sphygmomanometer cuff placed around the wrist to a pressure above 200 mm. Hg (Fig. 3). It did not occur in response to similar stimuli when the peripheral vessels were already constricted (Fig. 4). Similar stimuli produced no alteration in the pressure in the jugular vein with the subject in either the erect or

supine posture whether the peripheral blood vessels were in a dilated or constricted state.

The effect of a cuff placed around the arm distal to the needle and inflated by pressures beyond that of the systolic blood pressure for periods of 30–60 sec. was investigated in six subjects whose peripheral vessels were dilated. This was done in an endeavour to mimic the effects of peripheral vasoconstriction. In all instances the rapid inflation of the cuff was followed by a fall in pressure in the vein, which returned to the original level on release of the cuff pressure. This occurred both when the arm was below and when it was above the level of the manubrium sterni.

It was noted that the venous pulse diminished in amplitude during the period of vasoconstriction. This was especially evident in records obtained with the needle directed distally and is in substantial agreement with Hooker [1914], who noted the presence of a venous pulse when the peripheral vessels were dilated.

These observations indicate that, following a peripheral vasoconstriction brought about by a sensory stimulus, the venous pressure temporarily diminishes in the arms and legs whether the subject is erect or horizontal and irrespective of the relation of the limb to the body. They also show that the fall in venous pressure following a sensory stimulus is dependent on alterations in the circulation peripheral to the needle. Briscoe [1918] has observed that a cold stimulus causes a more marked fall in venous pressure in subjects with Raynaud's disease than in normal subjects.

(*c*) *Effect of respiration.* The effect of quiet respiration was studied in all subjects. In the supine position with the blood vessels dilated and the arm abducted to 90° from the body and placed below the level of the manubrium sterni, a fall of pressure occurred with inspiration and it was frequently prolonged until midway through expiration, at which time a return to the original level took place (Figs. 1 and 5). Evans [1932] also found that inspiration was usually associated with a fall in venous pressure, but Sewall [1906] is of the contrary opinion.

These variations in venous pressure were also present when the peripheral blood vessels were in a constricted state (Figs. 4 and 6). They were unaltered by a cuff occluding the circulation distal to the needle (Figs. 3 and 7). Similar changes in venous pressure occurred in the jugular and great saphenous veins when the subject was supine, but in the latter they were much less marked. If the arm were adducted to the side of the body in the supine position or if the arm hung dependent when the

subject was seated, the changes in venous pressure on respiration were frequently found to be in the reverse direction to those described above. This was attributed to elevation and compression of the subclavian vein by the first rib during inspiration. When the abducted arm was placed so that the point of insertion of the needle was above the level

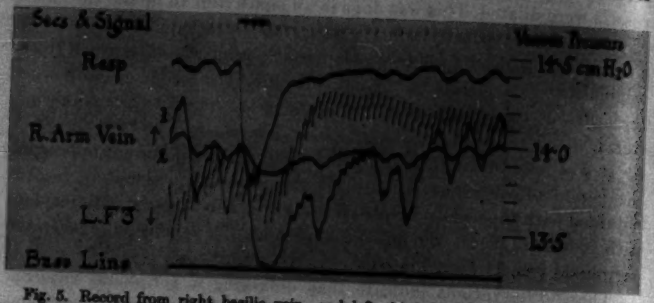

Fig. 5. Record from right basilic vein, and left third finger. Subject warm and peripheral vessels dilated. Arm below chest level. Quiet breathing and deep breath.

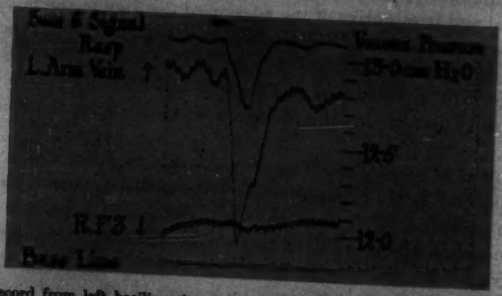

Fig. 6. Record from left basilic vein, and right third finger. Arm below level of chest. Subject cold and peripheral vessels constricted. Quiet breathing and deep breath.

of the manubrium sterni, no fluctuations in venous pressure in association with quiet breathing were observed. The minimal fluctuations to be observed in Fig. 8 are attributable to slight movement of the arm, altering the relationship of the level of the needle to that of the manometer. Similarly, no pressure changes were obtained when recording the pressure within the jugular bulb when the subject was in the sitting position.

These observations show that with the subject in the horizontal position a fall and rise of venous pressure is associated with the phases of respiration. These changes in venous pressure associated with quiet breathing are not influenced by the state of the vascular bed and circulation distal to the needle. If, however, the vein is above the level of

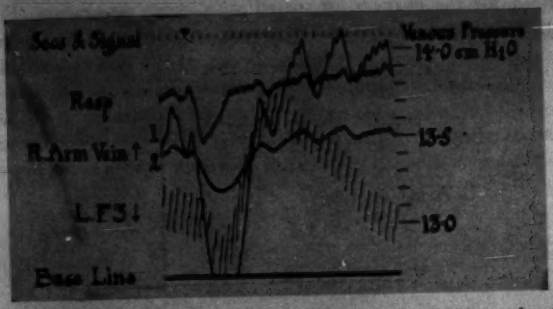

Fig. 7. Record from right basilic vein and left third finger. Subject warm and peripheral vessels dilated. Arm below level of chest and peripheral circulation occluded. Quiet breathing and deep breath.

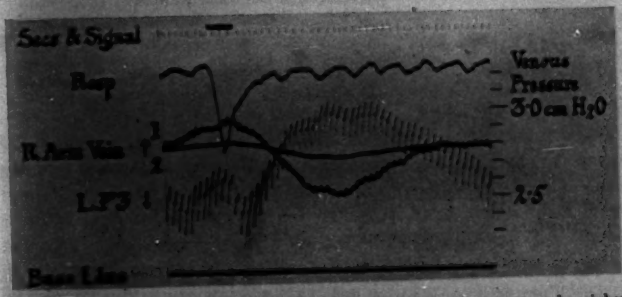

Fig. 8. Record from right basilic vein and left third finger. Subject warm and peripheral vessels dilated. Arm above chest level. Quiet breathing and deep breath.

the manubrium sterni these venous fluctuations disappear. These findings may be interpreted as showing that the changes in venous pressure are the result of changes in pressure proximal to the point of insertion of the needle; in other words, changes in intrathoracic pressure are only reflected distally along the venous channels when these are below the level of the heart.

The changes in venous pressure associated with a deep breath were in the same direction but of greater degree than those occurring with quiet breathing (Fig. 5).

Furthermore, alterations in posture influenced the fluctuations of venous pressure associated with a deep breath in a way similar to that described for the changes associated with quiet respiration (Figs. 8 and 9). The great saphenous vein, however, provided an exception in that

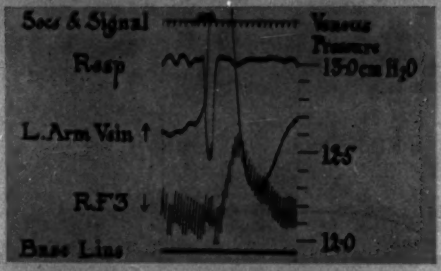

Fig. 9. Record from left basilic vein, and right third finger. Subject warm and peripheral vessels dilated. Arm below level of chest and adducted to side. Deep breath.

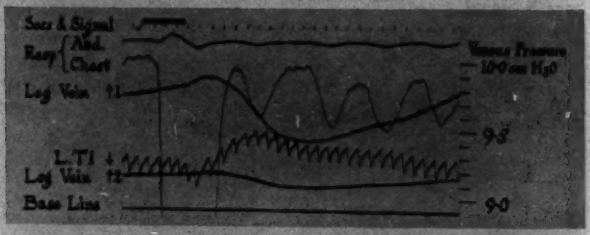

Fig. 10. Record from left saphenous vein, and left first toe. Subject warm and peripheral vessels dilated. Deep breath, chiefly chest movement.

a rise in venous pressure tended to occur during the inspiratory phase (Fig. 10). This rise in saphenous venous pressure was more marked when an abdominal breath was taken (Fig. 11). When the subject was in a warm state the venous pressure did not return to the original level during the expiratory phase, but gradually returned over a period of 30–40 sec. (Fig. 5). As it is known that following a deep breath a marked peripheral vasoconstriction occurs [Bolton *et al.* 1936], it is possible that this

delay in recovery of venous pressure resulted from the transient peripheral vasoconstriction. That this was indeed the case was shown as follows: First, it was shown that the slow return of the venous pressure to its base line was similar in all respects to the depression of venous pressure occurring after sensory stimuli; secondly, it could be modified in the same way by changes in the peripheral circulation. Thus the slow return was absent when the peripheral vessels were already in a constricted state (Fig. 6). It was also absent when the peripheral vessels were in a dilated state and the circulation distal to the needle was occluded by a cuff (Fig. 7). These procedures had no effect on the primary fall in venous pressure occurring with inspiration. As in the constricted state and in the dilated state with the peripheral circulation occluded, the

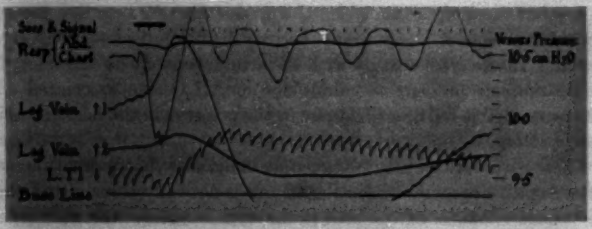

Fig. 11. Record from left saphenous vein, and left first toe. Subject warm and peripheral vessels dilated. Deep breath, chiefly abdominal movement.

fall in venous pressure still occurs, this fall must result from changes in pressure brought about by a mechanism proximal to the site of registration. This, as with quiet respiration, results from changes in intrathoracic pressure. It is apparent, therefore, that with a deep breath two factors are acting to cause the fall in venous pressure in a subject in whom the peripheral vessels are in a dilated state—one the fall in intrathoracic pressure and the other the peripheral vasoconstriction.

(*d*) *Effect of forced inspiration.* The effect of a forced inspiration against resistance was studied. The subject was requested to inspire through a tube which was attached to one limb of a U-tube containing mercury. For convenience the pressure has been expressed in cm. H_2O. It was assumed that the amount of negative pressure developed within the lungs was approximately equal to that shown on the U-tube manometer. If this be so, the actual negative pressure transmitted to the other contents of the intrathoracic cavity must have fallen not far short

of that observed in the manometer. The arm of a supine subject was abducted at right angles from the shoulder and placed below the level of the manubrium sterni so that a venous pressure of 20 cm. H_2O was recorded with the needle in the median basilic vein: the top of the fluid level in the manometer corresponded with the level of the manubrium sterni. When the subject produced a negative pressure in the region of 200 cm. H_2O for 15 sec., as described above, it was found that the pressure in the vein fell only 4 cm. H_2O. This new level corresponded approximately with the level at which the innominate vein joined the superior vena cava. When the subject slowly produced a negative pressure, the pressure in the median basilic vein fell correspondingly until the above level was reached. Further reductions in the intrathoracic pressure had no further effect on the venous pressure. With the arm elevated above the level of the manubrium sterni the production of a negative pressure by inspiring against a column of mercury had no effect on the venous pressure. In the erect posture with the arm dependent by the side a fall in intrathoracic pressure to minus 250 cm. H_2O was associated with either a negligible fall or a slight rise in the pressure in the median basilic vein.

These observations may be taken as indicating that a great increase in the negative pressure within the thorax is not fully transmitted along the venous channels of the arm if the vein be below the manubrium sterni, and not at all if the arm be above that level.

(*c*) *Size of veins.* During the course of these experiments it was observed that an increase in the size of the veins on the dorsum of the hand took place when the subject was warmed. This increase in size was compared to that produced by increasing the venous pressure when the subject was cold. In a thoroughly cold subject the diameter of an arm vein at a chosen spot 20 cm. below the level of the first rib measured 3 mm. A pressure of 50 mm. Hg was now added to the vein by inflating a cuff around the upper arm to 50 mm. Hg. This pressure was sustained for 5 min. The vein increased in size and measured 5 mm. After releasing the cuff the vein returned to its original size and the subject was then warmed by immersing the legs in water at 45° C. When sweating was well established and the fingers had become warm, the diameter of the same vein was 7 mm. That this increase in size of the vein might in part at least result from changes in temperature of the tissues surrounding the vein was shown in another experiment when the hand from which observations were being made was transferred from a water bath at 25° C. to a bath at 35° C. This resulted in an immediate increase in the

diameter of the vein. In order to control the factor of change in local temperature, the first experiment was repeated with the modification that the hand which was being observed was kept in a bath at 37° C. throughout the experiment and for half an hour before it commenced. The diameter of the vein when the subject was cold was 4 mm., while with a superadded pressure of 50 mm. Hg it measured 6 mm. After warming by immersing the legs in water at 45° C. it was 7 mm. These measurements were made with the hand at the same level. The latter observations indicated that the change in size of the veins concomitant with peripheral vasodilatation was not due to changes in local temperature. It was also apparent that this increase in diameter of the vein could not be explained by an increase in venous pressure, because a great increase in this pressure did not produce an increase in the diameter of the vein comparable with that produced by warming the subject.

(*f*) *Reaction of isolated segment of vein.* Finally, observations were made on the reactions occurring in a short segment of vein. A suitable vein free of tributaries for a length of 8 cm. was found at the wrist of one subject. Metal screw clamps fitted with small rubber pads were applied to the arm so as to compress the vein against the radius. Occlusion of each end of the chosen portion of vein was thus effected and a 20 gauge needle was inserted into the isolated segment. The contained blood was aspirated and replaced by a normal saline solution. The needle was then attached to the recording system which contained a column of fluid rising 20 cm. above the vein. That the lumen of the vein was effectively occluded at both the distal and proximal ends of the segment was shown by the maintenance of this pressure, and by the inability of a tourniquet around the upper arm to change the pressure in the segment. Two experiments were successfully performed on a subject who had previously been warmed to produce dilatation of the peripheral blood vessels. Stimuli, such as ice applied to the other arm, produced no changes in pressure in the vein nor did the taking of a deep breath. When, however, the individual was subjected to the widespread cold stimulus of a blast of cold air the pressure in the vein rose by 3–4 mm. H_2O and fell when the stimulus was stopped (Fig. 12). During this procedure the arm from which observations were being made was protected from the cold. This procedure was repeated four times in both experiments with the same results. Local cooling of the vein induced by applying ether to the skin overlying it, produced a rise of pressure of 2 mm. H_2O. The application of heat to the region of the vein caused a very slight fall in pressure. As an increase in pressure occurred in this

isolated segment on application of cold to the body, it is only possible to explain this rise by assuming that a constriction of the vein has been brought about by a nervous mechanism.

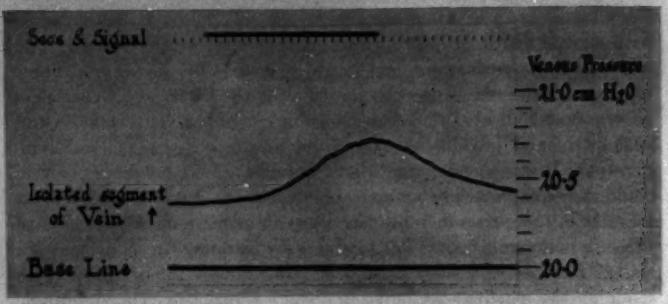

Fig. 12. Isolated segment of vein. Subject warm. Prolonged cold to body at signal.

Discussion

These results may now be discussed to demonstrate the parts played by the hydrostatic pressure, the capillary pressure and the size of the venous channels in determining the venous pressure.

Hydrostatic pressure. The hydrostatic element in venous pressure may be taken as consisting of the length of the column of blood up to the level at which the venous pressure becomes equal to the atmospheric pressure. This includes the vertical distance between the point of measurement and the highest point which the blood must reach on its return journey, plus the pressure in the right auricle at the point of entrance of the vein. The effect of altering the length of the blood column was shown by raising the arm, which caused the venous pressure to fall accordingly. A comparison in the erect posture of the venous pressure in the leg with that in the arm also showed the influence of this column on the venous pressure. A decrease in resistance to the flow by changing the pressure in the venous reservoir in the thorax was shown in the experiments on forced inspiration. It was demonstrated, however, that only a small part of the fall in intrathoracic pressure was transmitted to the veins outside the thorax. Indeed, despite a great fall in intrathoracic pressure the venous pressure could not be caused to fall lower than the hydrostatic pressure exerted by the column of fluid in the vein beyond its entrance to the thorax. This may be explained by the almost self-

evident fact that a negative pressure is not possible in readily collapsible tubes such as veins outside the thorax. Proof of this was found in the jugular vein in the erect posture and in the median basilic vein when the arm was elevated. Under these conditions the blood was being returned to the heart without having to overcome the hydrostatic pressure. A decrease in intrathoracic pressure in these circumstances had no effect on the venous pressure. As it has been shown that veins outside the thorax are incapable of transmitting a subatmospheric pressure, a negative intra-auricular pressure has no sucking action and so does not subtract from the pressure exerted by the column of blood in the vein. These findings adequately explain why a negative intrathoracic pressure was not transmitted to the median basilic vein in the seated subject with the arm below the level of the manubrium sterni. In this position the subclavian vein is above the level of the thoracic cage and so is beyond the influence of a negative change in intrathoracic pressure, but the effect of movement of the first rib persists. Furthermore, the above observations seem to invalidate the analogy which Clark *et al.* [1934] draw between the venous system and an elastic system whose contents are partly at a negative pressure.

Capillary pressure. The pressure moving the blood in the veins against the force of gravity is derived from the arterial pressure through the capillaries. In actual fact the capillaries transmit a slight excess of pressure above this minimum requirement. This slight excess was shown by a decrease in pressure in the median basilic and long saphenous veins when a peripheral vascular constriction occurred. Records from the jugular vein showed that this fall was not due to a diminution in hydrostatic pressure, as no fall in jugular pressure occurred, and the same result was brought about when a cuff was momentarily inflated distal to the needle in an arm vein. It may be presumed that the amount of the venous pressure in excess of the hydrostatic pressure represents the force required to drive the blood at the necessary rate along the narrow channels. It was also shown that the pressure transmitted from the capillaries was still present when the arm was elevated. Carrier & Rehberg [1923] ascribe the positive pressure found under such circumstances to the fact that the component of the resistance due to friction will be increased by the collapse of the veins and the increased velocity of the blood flow. It may more simply be regarded as being determined by the amount of pressure required to keep the veins patent.

During the course of these experiments the subjects endeavoured to remain motionless. In this way the pumping action of muscular

contraction and the influence of the valves were excluded. Under such conditions the venous pressure at all points of recording showed that the driving force derived from the capillaries was sufficient to return the venous blood to the heart. This point was strikingly shown in the case of the lower limb in which the venous pressure measured in cm. H_2O at the ankle was equivalent to the vertical distance below the third interspace. From this observation it was also apparent that the intra-auricular contents above that level were at a subatmospheric pressure. If allowance is made for the difference in specific gravity of blood and the saline solution one may conclude that the zero pressure level in the right auricle is approximately at the fourth interspace in the erect subject. During quiet breathing very small alterations took place in the venous pressure. It appears, therefore, that the ordinary fluctuations in intrathoracic pressure are of relatively little importance in returning the blood to the heart and that an adequate pressure is obtained from the capillaries to accomplish this purpose. The negative intra-auricular pressure in the erect position does however remove a great hydrostatic load from the veins of the lower half of the body, and when the auricular pressure is positive, as in the recumbent position, a lowering of the hydrostatic pressure will occur with each inspiration. This may not be called a true suction effect. Within these limitations the negative intra-auricular pressure deserves the term "vis a fronte" applied to it by Bedford & Wright [1924].

Capacity of the venous channels. The observations that a fall in venous pressure occurred with a transitory peripheral vasoconstriction suggested that this was not associated with a constriction in the venous channels. However, the fact that no alteration in venous pressure took place during the dilatation of the peripheral blood vessels despite the increased blood flow and more direct communication between the arteries and veins showed that the venous channels must have increased in capacity. That such an increase does occur is an every-day observation. This increase in size was not due to an increase in venous pressure, for there was no change in pressure, and a marked rise in venous pressure in the cold subject did not produce an increase comparable to that caused by warming the subject. Lewis & Landis [1930] have previously observed the effect of local temperature on the size of the veins. However, the fact that an increase in diameter of the veins takes place on warming the subject while the local temperature is kept constant shows that the dilatation is not wholly dependent on the change in local temperature. It has been shown that active constriction of the superficial veins may

take place in response to a sufficiently cold stimulus elsewhere to the body. The constriction following such a stimulus must be the result of a nervous mechanism operating to bring about constriction of the muscular walls of the vein. Means [1935] has also obtained evidence in the human of a nervous mechanism controlling the veins, and Lewis & Landis [1930] observed an increase in size of the veins following sympathectomy. Moreover, it would seem that a nervous action would be the probable mechanism underlying the maintenance of a constant venous pressure during peripheral vasodilatation. Inasmuch as the veins increase in size following sympathectomy, it may be suggested that the alteration in size of the superficial veins produced by warming the body is in part at least due to inhibition of the activity of sympathetic vasoconstrictor fibres supplying the musculature of the veins.

Summary

Venous pressures have been recorded from the jugular bulb and median basilic and long saphenous veins.

A comparison has been made between the pressure in the supine and erect postures and the effects of altering the position of the limb have been noted. In the supine position the venous pressure at all points of recording reached to the level of the manubrium sterni, while in the erect posture the pressure in the long saphenous vein reached to the third intercostal space, in the median basilic to the level of the first rib, and in the jugular bulb it approached zero.

The influence of respiration on this pressure has been described and it has been shown that subatmospheric pressures are not present in veins outside the thorax.

The influence of changes in capillary pressure has been demonstrated by recording a fall in venous pressure synchronous with a peripheral vasoconstriction. It has also been shown that a small component of the total venous pressure is utilized to return the blood at an adequate rate.

The adjustment in calibre of the veins of the limbs during vasodilatation has been noted and this has been ascribed in part to a nervous mechanism.

The authors desire to express their gratitude to Dr E. Arnold Carmichael for his invaluable advice and assistance.

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ANTAGONISM BETWEEN PROGESTERONE AND THE SYNTHETIC ŒSTROGENIC SUBSTANCE, TRIPHENYL ETHYLENE

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It is well known that the actions of the hormone of the corpus luteum on the rabbit's uterus can be antagonized by œstrin. The specific progestational reaction of the endometrium is no longer produced when suitable quantities of œstrin are administered together with a corpus luteum extract or with progesterone, and the desensitization of the uterine muscle (i.e. inhibition of the reaction to the oxytocic hormone of the posterior lobe) is also reversed to some extent [Robson & Illingworth, 1931; Leonard *et al.* 1932; Courrier, 1936; Robson, 1936].

In view of the discovery by Dodds and his co-workers that certain comparatively simple synthetic substances possess œstrogenic properties [Dodds *et al.* 1937] it became of interest to determine whether such substances would also antagonize the specific effects of the luteal hormone. The substance, triphenyl ethylene, was used in these experiments as it is readily available and has a well defined œstrogenic activity [Robson & Schönberg, 1937].

Methods

Mature female rabbits of various breeds weighing about 2·0 kg. were used. The animals were spayed by the dorsal route and then received seven or eight daily subcutaneous injections of 10μg. of œstrone in oil, the first injection being given on the day after the operation. This preparatory treatment was followed by injections of progesterone and triphenyl ethylene which were spread over a period of 4 days. The substances were dissolved in oil and administered subcutaneously twice daily. All animals received a total amount of 0·75 mg. of progesterone in order to allow comparison with the quantitative results previously obtained on the antagonism of œstrone and progesterone. On the day after

the last injection the animals were operated on under ether anæsthesia; one uterine horn was removed for histological examination and investigation of the reactivity of the muscle to oxytocin *in vitro*, while the reactivity of the uterine muscle *in vivo* was determined with the other horn. The experimental details have been previously described [Robson, 1935]. A progestational proliferation of 3·0 is similar to that seen at the height of pseudo-pregnancy while 1·0 represents the minimum clearly definable proliferation.

I am indebted to Dr Neumann of Messrs Schering and Co. for the supply of progesterone in solution in oil (proluton); to Dr White, of Parke, Davis and Co., for a purified sample of the oxytocic hormone of the posterior pituitary containing 10 units of oxytocic activity and 0·6 unit of pressor activity per c.c. (this will be referred to as pitocin in this paper); and to Mr Bennet of British Drug Houses Ltd., for the supply of triphenyl ethylene.

Results

The results are shown in Table I, which also includes experiments previously obtained with progesterone only, in order to allow of comparison. In addition the present supply of progesterone was tested on two rabbits (919, 938) under the experimental conditions described above,

Table I. The condition of the endometrium and uterine muscle. All animals were spayed and then treated with œstrone for 7 or 8 days. In the next 4 days they received 0·75 mg. of progesterone plus various doses of triphenyl ethylene. The uterus was examined on the day after the last injection.

	Injections		Progestational proliferation (0–3)	Response to oxytocin. Min. effective dosage		Spontaneous activity	
Animal no.	Progesterone mg.	Triphenyl ethylene mg.		*In vitro* units per 100 c.c.	*In vivo* units injected	*In vitro* cm. movement	*In vivo* cm. movement
919	0·75	0	2·5	—	—	—	—
938	0·75	0	3·0	—	—	—	—
647	0·75	0	2·5	5·0	1·0	13·0	0·4
624	0·75	0	3·0	5·0	0·5	0·9	0·7
633	0·75	0	3·0	1·0	1·0	7·0	0·7
920	0·75	20	2·0	1·0	1·0	1·0	0·2
941	0·75	20	2·0	0·1	0·1	3·8	0·4
908	0·75	40	1·0	1·0	1·0	2·5	0·1
912	0·75	40	1·0	1·0	1·0	10·0	0·2
856	0·75	80	1·0	1·0	1·0	5·0	0·2
907	0·75	80	Nil	1·0	1·0	17·0	2·0
900	0·75	160	Nil	0·1	0·1	17·0	1·7
901	0·75	160	Nil	0·3	0·3	20·0	3·5
939	0·75	160	Nil	0·03	0·1	19·0	1·2

and 0·75 mg. was found to give a progestational proliferation of 3·0 and 2·5 respectively. The figures given for spontaneous activity *in vitro* represent for each animal the mean of two experiments.

It will be seen that 160 mg. of triphenyl ethylene completely antagonize the progestational action of 0·75 mg. of progesterone; smaller doses produce a partial antagonism. The desensitization of the uterine muscle can also be prevented when triphenyl ethylene is injected together with progesterone. The motility of the uterus *in vivo* is increased when triphenyl ethylene is injected with progesterone and there is some suggestion that the same applies to the rhythmic activity *in vitro*.

Discussion

The results show that the action of progesterone on the uterus can be antagonized not only by a naturally occurring œstrogenic hormone but also by a synthetic substance possessing œstrogenic properties. This applies not only to the progestational proliferation of the endometrium but also to the desensitization of the uterine muscle, as measured both *in vitro* and *in vivo*. Moreover, triphenyl ethylene increases the motility of the uterus *in vivo* (and probably *in vitro*) even though progesterone is administered simultaneously.

The results suggest that the inhibition by œstrogens of the action of progesterone is not dependent on similarity of chemical structure, as suggested by Pincus [1937], since triphenyl ethylene possesses a structure completely different from that of œstrone and progesterone.

It is interesting to consider quantitatively the antagonism between progesterone and œstrogenic substances. Fig. 1 shows the relation between the progestational effects on the endometrium and the quantity of progesterone given, and the influence of the addition of two different doses of œstrone, most of the necessary data being taken from results previously published [Robson, 1936]. It will be seen that as the dose of œstrone is doubled the dose of progesterone has also to be doubled in order to keep the effects on the endometrium constant.

In order to compare the effectiveness of œstrone and triphenyl ethylene in inhibiting the progestational action of progesterone, the endometrial effects produced by combination of a constant dose of progesterone (0·75 mg.) and various doses of œstrone and of triphenyl ethylene respectively have been plotted in Fig. 2. The doses of the latter two substances are measured in biological (mouse) units in order to facilitate a comparison of their effects. It has been assumed that 0·1 μg.

œstrone = 0·5 mg. triphenyl ethylene = 1 mouse unit. This appears to be justifiable since it has been shown [Hain & Robson, 1936] that 0·1 μg.

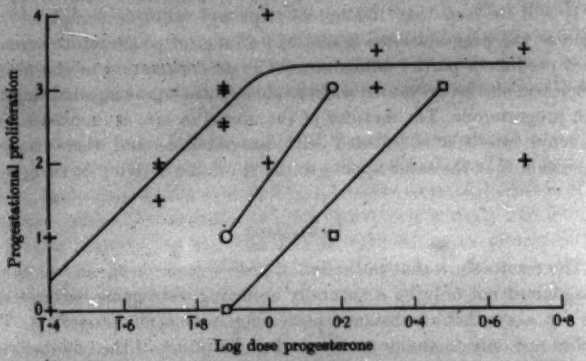

Fig. 1. The effect on the endometrium (progestational proliferation) of various doses of progesterone, alone or in combination with 5 or 10 μg. of œstrone. + = No œstrone. ○ = 5 μg. œstrone. □ = 10 μg. œstrone.

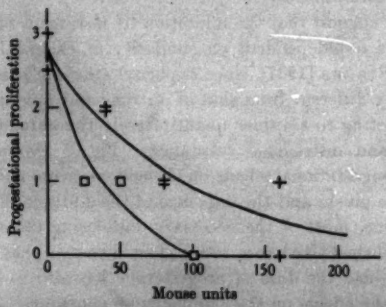

Fig. 2. Comparison of the antagonistic action of œstrone and of triphenyl ethylene to the effect of progesterone on the endometrium. + = 0·75 mg. progesterone + triphenyl ethylene [1 M.U. = 500 μg.]. □ = 0·75 mg. progesterone + œstrone [1 M.U. = 0·1 μg.].

œstrone injected in four doses in oil in ovariectomized mice produces full cornification in 60 p.c. of animals, while the administration of 0·5 mg. triphenyl ethylene under exactly similar experimental conditions also produces cornification in 60 p.c. of mice (unpublished results). Fig. 2

shows that, as regards the inhibition of the progestational action of progesterone, œstrone is about three times as effective (per biological unit) as is triphenyl ethylene.

SUMMARY

The effects of progesterone on the endometrium and muscle of the rabbit's uterus can be antagonized by the synthetic œstrogenic compound triphenyl ethylene. The results on the endometrium are quantitatively presented and the activity of triphenyl ethylene is compared with that of œstrone. It is emphasized that, in spite of the great difference in chemical constitution, both the naturally occurring œstrogen and triphenyl ethylene can inhibit the specific effects of progesterone.

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THE EFFECT OF ADAPTATION ON DIFFERENTIAL BRIGHTNESS DISCRIMINATION

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The sensitivity of the human eye to changes in brightness has generally been investigated under conditions which gave approximate adaptation to the intensity at which the differential threshold was being tested. If the brightness of the field be I and a threshold change in its brightness ΔI, then plotting $\Delta I/I$ against I, for different values of I, gives the familiar curve showing gradual decrease of $\Delta I/I$ with increasing I. This decrease becomes smaller until, according to the most recent investigators [Lowry, 1931; Guild, 1932; Steinhardt, 1936; Stiles, 1929], at high illuminations $\Delta I/I$ reaches a nearly steady value. "Weber's law", first applied to light intensities by Fechner, was a statement of such constancy of $\Delta I/I$, and thus over the higher range of intensities it is approximately obeyed.

The smallest value attained by ΔI is 0·5–2 p.c. of I, depending to some extent on the method of measurement. At progressively lower intensities the ratio of ΔI to I increases till it becomes about 100 p.c.; this is in the neighbourhood of the absolute threshold, and measurements at still lower values become impossible. We shall return later to the discussion of this curve; at present it is interesting to note that a rise in differential threshold at very high illuminations was obtained by König & Brodhun [1889], but has not been found in so marked a form by later investigators [Guild, 1932; Lowry, 1931; Steinhardt, 1936], who suggest that the rise in threshold found in the earlier experiments was due to inadequate adaptation of the eye to these higher intensities.

There is ample evidence that keeping the eye for an hour or more in darkness leads to an increase in its absolute sensitivity, and that conversely exposure to a high illumination produces a decrease in absolute sensitivity. For instance, the absolute threshold becomes progressively lower while the eye is in darkness; and on the other hand the absolute

threshold is raised by exposure to high illuminations [Nutting, 1920]. Further, if after a period in darkness the eye is exposed to a bright surface, the apparent brightness of this surface decreases during the first few minutes. These phenomena indicate processes of dark and bright adaptation in the eye, and raise the question as to whether differential sensitivity is also dependent upon the adapting illumination. This problem was investigated by Wright [1935, 1936] using monochromatic light, over a fairly small range of adapting and testing intensities. He exposed the eye for some minutes to a field having a certain brightness and subsequently tested its differential sensitivity by brief exposures to a bipartite field having, as a rule, a different brightness. He showed that under such conditions it was possible to maintain a state of nearly complete adaptation to the one intensity and to obtain a measure of the differential sensitivity at the other. He found that the differential threshold at a given "test" intensity was very little affected by the adapting intensities he used, and that differential brightness sensitivity could not, therefore, be a function simply of the state of adaptation of the eye.

Mellone & Rawdon-Smith [1935, unpublished], employing white light, again over a limited range of intensities, were able to show that adaptation had a small effect upon the differential threshold. If the eye was tested at any intensity other than that to which it had been adapted, the differential threshold was slightly higher than when the testing and adapting illuminations were equal.

Similar conclusions were reached by Cobb [1916] and by Lythgoe & Tansley [1929] using a dark or bright surrounding field in place of successive adapting and testing conditions. In the latter's experiments, critical flicker frequency was the criterion employed.

It seemed likely that an investigation over a larger range of intensities might indicate how far the differential brightness threshold is dependent on, or independent of, the state of adaptation of the eye.

Apparatus

The present apparatus was designed to provide "adapt" and "test" fields of any brightness from those in the neighbourhood of the absolute threshold of the dark adapted eye, up to a maximum of 4000 equiv. f.c.[1]

[1] By this is meant a brightness equivalent to that of a perfectly diffusing white screen with an illumination of so many foot-candles upon it. In view of Stiles & Crawford's [1933] recent evidence that retinal illumination is not directly proportional to pupil area, except for very low illuminations, the photon loses much of its value as a unit, and it seems wiser to use this very familiar one, which is equivalent to 1·07 millilamberts.

These require to be independently controllable, so that any "adapt" brightness can be followed by any "test" brightness, and the rapidity of readaptation makes it essential that the change from "adapt" to "test" should be nearly instantaneous. It is necessary to adapt a larger area of the retina than that which is tested, in order that the area to be tested subsequently should be completely light-adapted. It is important, also, to use both a large test field, to study mixed rod-and-cone vision, and a small field to obtain nearly pure cone vision. It is important that the "test" and "adapt" fields should be illuminated by the same light source to avoid changes in colour due to differences in filament temperature and ageing of two bulbs.

For the test field, an illumination flickering with a constant frequency (2 c./sec.) but with variable amplitude seems preferable to a bipartite field or a large field containing a small area of slightly different intensity. Accurate discrimination under these latter conditions appears to demand small, continuous eye movements [Helmholtz, 1866; Hecht, 1935] which are not required with a flickering field. Further, the flicker method is found to give values of $\Delta I/I$ comparable with those obtained by other methods, and has the advantage of providing a nearer approach to the actual "sensational increment" upon which most of the theories of intensity discrimination, from Fechner to Hecht [1935], have been based. Further, it does not demand accurate fixation and accommodation by the eye of a test patch or boundary, which might prove difficult during the rather short exposures necessary to prevent the eye from recovering from the "adapt" illumination and becoming adapted to the "test" illumination, during the course of a single exposure.

In the apparatus, the field is provided by a lens, the eye being placed at one conjugate focus; at the other conjugate focus is a clear-glass 100 W. bulb with a concave mirror behind it (the Maxwellian view). The lens appears uniformly filled with light. The light passing directly from the filament through the lens provides the illumination I; the light reflected from the mirror and thence passing through the bulb and lens provides the additional fraction ΔI, which is varied by a diaphragm between the mirror and the bulb. A double filter frame near the lens enables any pair of "adapt" and "test" illuminations to be secured.

DETAILS OF APPARATUS

A 200 V. 100 W. gas-filled clear bulb *a* (Fig. 1) illuminates a ground-glass disc *c* through a stop *d*. A lens *h* of 18 mm. diameter and 16 mm. focal length, 23 cm. from the aperture stop *d*, forms an image of it on the pupil of the eye *l*, thereby producing a uniformly bright

field whose angular size is determined by a stop at *g* (the principal focus of *h*). Stops giving fields of 45, 36 and 1½° may be inserted here. The image of *c* on the pupil is 3 mm. in diameter and thus largely eliminates the influence of changes in pupil size on retinal illumination, as pointed out by Hecht [1937]. A double frame *c* holds, in each half, either two 25 mm. square Wratten neutral filters, mounted in B glass, or one filter and a clear glass square, or a blank stop. By these means any given illumination for adapting and testing may be obtained. A shutter *f* cuts out the light during the change over of filters and field stops. A glass cell at *b*, with cold water circulation, cuts off the greater portion of the infra-red radiation from *a* and so protects the subject's eye and the filters, as well as cooling the interior of the apparatus.

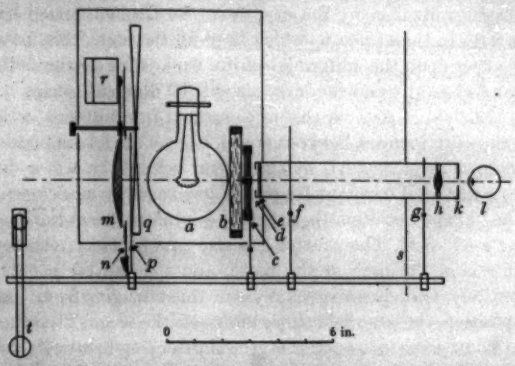

Fig. 1. *a*, 100 W. clear bulb; *b*, water cell; *c*, double filter frame; *d*, aperture stop; *e*, ground-glass screen; *f*, shutter; *g*, field stop; *h*, lens; *k*, cap; *l*, eye; *m*, concave mirror; *n*, diaphragm with scale and pointer; *p*, shutter; *q*, sector disk; *r*, motor to drive sector disk; *s*, screen to intercept stray light; *t*, key arm.

Behind the bulb is a concave mirror of 7·5 cm. focal length and 7 cm. diameter, so placed that the aperture stop *d* and the filament of the bulb are conjugate foci. The image of the filament cast by it on the ground glass thus acts as an additional source of illumination for the lens *h*, and adds 200 p.c. to the illumination of the field as seen by the eye.

In front of this mirror are an adjustable diaphragm *n*, a shutter *p* and a sector disk *q*, the latter being driven off a small synchronous motor so as to add the illumination due to the mirror to that directly from the lamp in the form of a flicker of constant frequency (2 c./sec.). The diaphragm *n*, constructed of two L-shaped bars with links on the principle of parallel rulers, provides a rectangular aperture variable from 0 to 5 cm. side. The shutters *p* and *f*, the filter frame *c* and the field stop *g* are all connected by links to levers on a rod rotated by a key arm and provided with return springs, so that all operate together on depressing the key.

The whole is provided with a metal case to prevent the escape of light, thus enabling the room to be almost completely dark. The instrument is placed on a table of adjustable height and provided with a head rest for the subject.

The apparatus has the following characteristics. It provides an adapting field of 45° and of any brightness between 1/2500th and 4000 equiv. f.c. Test fields of 1½ and 36° are also obtainable over the same range of illuminations. All possible combinations of pairs of illuminations over this range can be produced. The adapting field is made larger than either of the test fields to secure complete adaptation of the area to be tested, and to prevent small eye movements from exposing unadapted areas of the retina at the margin of the test field.

The use of a variable diaphragm in front of the mirror causes variation in the angle subtended by the rays from the filament image on *e*, and consequently in the degree to which they fill the lens. This, however, is without effect upon the uniformity of the field owing to the diffusion by the ground glass at *e* and the large size of the filament image.

The test field is also capable of a gradual intermittent variation in brightness—any increase between 0 and 200 p.c. of I being added twice per second, so as to give ΔI, by the mirror behind the sector disk. This provides a flicker of constant frequency but variable brightness change.

In the "adapt" position the field stop *g* is out of the optical path, thus exposing a 45° field. The shutter *f* is also moved away, one set of the filters in *c* is in the path of the beam, and the shutter *p* cuts off the mirror entirely. On depressing the key arm, thus changing from "adapt" to "test" position, the large field stop *g* intersects the beam. The other pair of filters in *c* now come into operation, the shutter *f* momentarily darkening the field while the change takes place. The shutter *p* also moves away, exposing the mirror which adds an illumination determined by the aperture of the adjustable diaphragm *m*, every half second, i.e. when the sector disk exposes it. All moving parts are damped and silenced by felt or rubber stops, and the change from "adapt" to "test" positions takes less than 0·1 sec., involving only the depression of one key. Between different groups of test and adaptation stimuli this key can be locked in an intermediate position by a catch, so that the shutter *f* cuts off the light from the subject and so enables the sets of filters to be changed for the next series of readings, without moving the subject's head. The link moving the filter frame can be detached to enable it to remain in the "adapt" position while the other shutters move, so that equal illuminations for "test" and "adapt" positions are obtainable without duplication of filters.

Calibration. The calibration of the diaphragm scale *m*, which was done directly both for Log $\Delta I/I$ and for ΔI as a percentage of I, was accomplished by a selenium oxide photocell of the generator type clipped over the eyepiece. The diaphragm scale was calibrated with no filters inter-

posed, and checked with the 1/10th transmission filter in position. (The insertion of filters was without effect upon the relative calibration of this diaphragm. The transmissions of the Wratten filters were sufficiently close to their theoretical values, for use in obtaining the range of brightnesses I, for adapting and testing.) The photocell was attached to a microammeter previously calibrated with the cell over the same range of illuminations (4000–12,000 equiv. f.c., and 400–1200 equiv. f.c.), using a diffusing screen illuminated by a 500 W. bulb at various distances, the illuminations being calculated using the inverse square law. The absolute calibration was secured by illuminating a screen subtending the same angle as the field of the instrument, by means of the same 500 W. bulb. The photocell was fitted with a mask having an aperture equal to the exit pupil of the apparatus. This lamp was in turn calibrated by a 20 c.p. N.P.L. substandard lamp. The cell was checked at intervals by a fixed illumination to avoid errors due to "fatigue" at high illuminations. Great accuracy of absolute calibration in an instrument such as this is, unfortunately, somewhat difficult. The maximum error involved in this method of calibration, however, is negligible for the purposes for which the apparatus was designed.

Experimental procedure

The subject was seated in a dark room with a shade over the eye which was not being employed. His head was fixed by means of a rest in such a position that the image of the aperture stop of the apparatus (the Ramsden circle) was sharply focused and centrally placed upon the subject's eye.

The "adapt" and "test" filters for the first series of readings were then inserted and the subject's eye exposed to the adapting illumination for a time sufficient to ensure the attainment of a practically steady state. This period was found by measurements of the absolute and differential threshold at different times during the course of adaptation, and by the time required for complete disappearance of the after image of the test field upon the larger adapting field. The major portion of the adaptive process appeared to be complete in 20 min. for dark adaptation and 2 or 3 min. for adaptation to the highest intensities. Considerably longer periods would have been preferable, but the importance of obtaining numerous readings without unduly fatiguing the subject rendered them difficult in practice.

At the end of this period the experimenter pressed the key for a period of 2 sec., thus changing from adapt to test conditions. The subject

then reported whether or not the test field had appeared to flicker. After 2 sec. the adaptation condition was restored for a period of 1–5 min., the shorter period being employed where the "test" and "adapt" illuminations were equal, and the longer for recovery of dark adaptation after a very bright test exposure. This cycle of operations was repeated with the diaphragm *n* set at progressively larger or smaller apertures, thus permitting a determination of the point of appearance and disappearance of flicker. This gave a measure of the differential brightness threshold by the method of limits.

Tests of the differential threshold at the adapting illumination, and of the absolute threshold, immediately before and after exposure to the test illumination, showed that (for most combinations of test and adapt illuminations) 2 sec. exposure to the test illumination did not seriously change the state of adaptation of the eye. At the same time, it provided adequate time for judgment, both in the opinion of the subjects and as assessed from the consistency of the results. When, however, the fully dark-adapted retina was exposed to extremely bright test fields a considerable amount of bright adaptation was inevitable. It will be appreciated that, since the time required for almost complete dark adaptation is of the order of an hour, it is impossible to obtain many readings under these particular conditions, without tiring the subject to such an extent as to render his results unreliable. Sufficient readings have been obtained, however, to indicate the general trend of the curves under this most difficult condition of high intensity discrimination with the dark-adapted eye.

After each series the key arm was locked in the mid-position and the filters for the next series inserted. Illuminations below 1/10,000 of the maximum were obtained by using two filters, one in front of the other.

The whole experiment was performed with 36 and $1\frac{1}{4}°$ test fields, giving mixed rod and cone vision, and nearly pure cone vision, respectively. Series of readings were taken in two ways: (1) with fixed test against different adapting illuminations, and (2) with fixed adapting against different test illuminations.

In no case did anyone act as subject for more than an hour on any one day. As it was thus impossible to perform the whole experiment at one time, several series were repeated on different days to check the constancy of the results. These were found to be reproducible within the limits imposed by other sources of error. Two subjects were used in the main experiment, both experienced in making such judgments. One of the subjects (A.F.R.S.) has myopia and some astigmatism.

Results

The results of this experiment can most easily be expressed in the form of graphs.

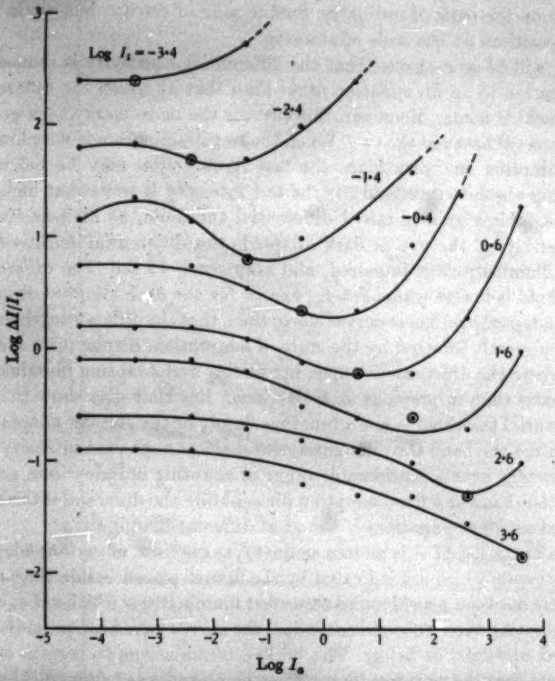

Fig. 2. Test field = 36°. Ordinate: log $\Delta I/I_{test}$. Abscissæ: log I_{adapt} for log I_{test} = 3·6, 2·6, 1·6, 0·6, −0·4, −1·4, −2·4, −3·4, respectively, reading upwards. Equal "test" and "adapt" illuminations are indicated by circles round certain points. All curves above the lowest are moved upwards successively by 0·5 on the ordinate scale, to avoid confusion. True ordinate values of each may thus be read by subtracting 0 from the ordinates of the lowest curve, 0·5 from those of the second, 1 from those of the third, etc. Subject K.J.W.C.

In Fig. 2 the differential threshold is plotted in terms of log $\Delta I/I_t$ against I_a, where I_t and I_a are testing and adapting illuminations respectively. Each curve shows the values of this threshold at the illumina-

tions noted beside it. The broken lines terminating some of the curves indicate that the next test illumination employed was below the absolute light threshold under the conditions of adaptation then obtaining. All the curves above the lowest are raised successively by an amount equal to 0·5 on the scale of ordinates, for the sake of clarity, but are in their true positions on the scale of abscissæ.

It will be seen at once that the differential sensitivity is reduced by adaptation to an illumination other than that at which the differential judgment is made. Moreover, this effect is the more marked the greater the interval between the two. When the test illumination is very low and the adapting one very high, the test illumination may be below the existing absolute threshold. If the test intensity is somewhat higher, it may exhibit a greatly raised differential threshold, as high as 100 p.c. Conversely, if the eye is dark adapted, the differential sensitivity at high illuminations is impaired, and may reach 15 p.c. The differential threshold is lowest where $I_t = I_a$, except for the dark-adapted or nearly dark-adapted eye. These curves show, then, that the differential threshold is very greatly affected by the state of adaptation, displaying a marked rise where the difference between the testing and adapting illuminations is greater than in previous investigations. But they also show that the differential threshold is not a function simply of the state of adaptation; for on the one hand the differential threshold at a selected intensity may be constant over a considerable range of adapting illuminations, and on the other hand at a fixed adapting illumination the differential threshold may show great variations if tested at different illuminations.

In Fig. 3, $\log \Delta I/I_t$ is plotted against I_t as abscissa, at various adapting brightnesses which are indicated by the figures placed beside the curves. A circle has been placed round those test illuminations which are equal to the adapting ones. To avoid confusion, the curves have been progressively shifted upwards, as before. The broken terminations to certain curves indicate that the next test illumination employed was below the absolute threshold under the existing conditions of adaptation. It may be noted that the five curves between $\log I_a = 0{\cdot}4$ and $\log I_a = 3{\cdot}6$ may be approximately superimposed if the "equal test and adapt" points are made coincident. The curves obtained with adaptation to an illumination below 10^{-2} equiv. f.c. are, however, of a totally different character.

In Fig. 4 similar curves are given, showing the differential sensitivity with a $1\frac{1}{2}°$ "rod-free" field. Here the ability to discriminate intensities below approximately 10^{-2} equiv. f.c. is lost, and the lower curves are consequently absent; they coincide within the limits of error with those

at 10^{-2} equiv. f.c. adaptation. Further, the curves do not show any marked rise in differential threshold when the eye is dark adapted and

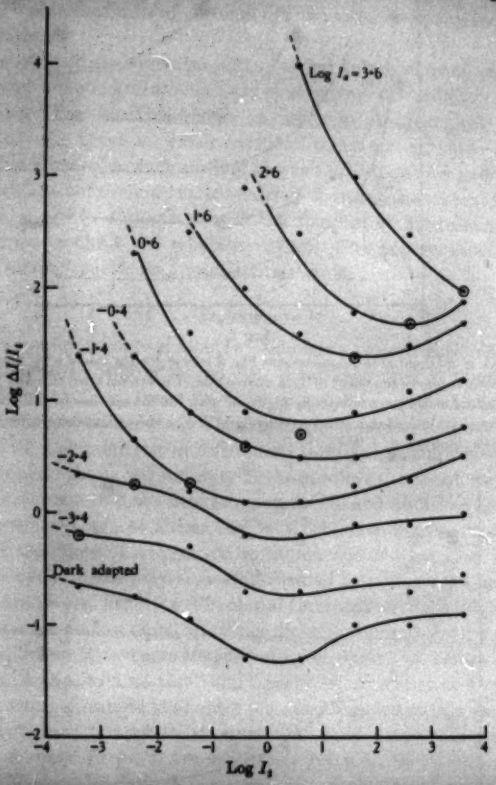

Fig. 3. Ordinate: log $\Delta I/I_t$. Abscissa: log I_t for a 36° test field, at dark adaptation (in the lowest curve) and at log $I_a = -3{\cdot}4, -2{\cdot}4, -1{\cdot}4, -0{\cdot}4, 0{\cdot}6, 1{\cdot}6, 2{\cdot}6, 3{\cdot}6$, respectively, reading upwards. All curves above the lowest are moved upwards successively by 0·5 on the ordinate scale, as before (see Fig. 2). Equal "test" and "adapt" illuminations are indicated by circles round certain points. Subject K.J.W.C.

tested at high intensities; the rise found under these conditions with "mixed" vision, which is accompanied by extreme sensations of dazzling, may also, therefore, be attributed to rod vision.

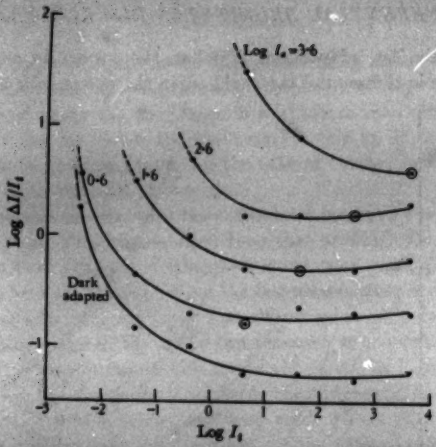

Fig. 4. This is obtained in the same way as Fig. 3 except for the use of a 1½° test field. The lowest curve shows the effect of dark adaptation. Curves between this and log I_a=0·6 are omitted as they are effectively identical with the lowest curves. All curves above the lowest are moved upwards successively by 0·5 on the ordinate scale, for the sake of clearness. Subject K.J.W.C.

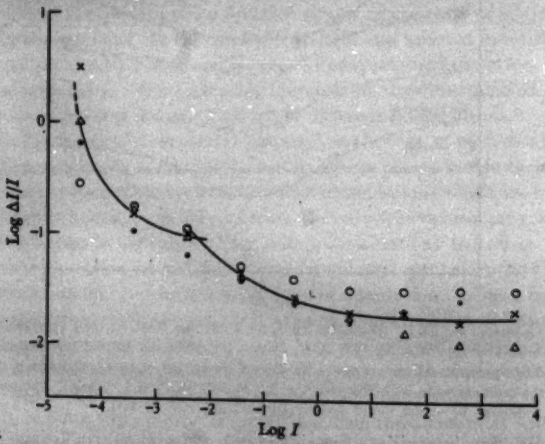

Fig. 5. Log $\Delta I/I$ is plotted against log I, using four subjects, so as to show the differential sensitivity when "test" and "adapt" illuminations are always equal. Test field 36° diameter.

These results were obtained on two subjects; while several series which were repeated with two other subjects gave curves which were identical within the limits of personal differences in sensitivity and day-to-day variations.

Series of readings were also taken on four subjects, using only equal testing and adapting illuminations, thus giving the ordinary "Weber law" curve (Fig. 5). This shows an inflexion at approximately 10^{-2} equiv. f.c.—the point at which rods and cones are supposed to interchange functions; the work of Steinhardt [1936] on this point is thus confirmed. The curve shows little tendency to rise at the highest intensity available, again in agreement with the findings of Steinhardt. This curve served to check the reliability of the other curves, whose "equal test and adapt" points lie approximately on it.

Discussion

These results indicate that adaptation has a very marked effect upon the differential sensitivity of the eye. When measured at illuminations widely different from those to which the eye is adapted, differential sensitivity is greatly reduced. There is ample evidence that we are here dealing with a genuine rise in differential threshold and not merely with an effect due to the close proximity of the absolute threshold, for in most cases subjects reported the field as being visible and unflickering when it would have been seen to flicker had they been less bright adapted. The extent of the effect of adaptation to illuminations other than those at which the eye is tested may be best illustrated by giving a few examples. Using the large test field, the differential threshold at a test brightness of 4000 equiv. f.c. and an equal adapting illumination is about 2 p.c.; after dark adaptation it is approximately 15 p.c., tested as before at 4000 equiv. f.c. Again, with a "test" and "adapt" illumination of 4 equiv. f.c. the differential threshold is about 2 p.c., and remains at this value after dark adaptation; but after adaptation to 4000 equiv. f.c. (tested as before at 4 equiv. f.c.) it is approximately 100 p.c.

The differential threshold measured with equal "test" and "adapt" illuminations shows no significant rise even at 4000 equiv. f.c. This confirms the results of Lowry [1931], Guild [1932] and Steinhardt [1936]. They explained the rise found by previous investigators at lower values as being due to inadequate adaptation of the eye to these high intensities. The present results perhaps strengthen this interpretation, for it is shown that insufficient bright adaptation does, in fact, cause just such a rise in differential threshold at these illuminations, which is absent

when adaptation has been more fully attained. It remains possible, however, that there is a slight rise at even higher intensities, as found by Steinhardt. Steinhardt doubts whether this rise is genuine, since it is not found with small test fields. It may be argued that this is not a valid reason for discarding the rise, for the present results show that, with a small centrally fixated field, bright adaptation is much less important. Stiles [1929], using a test patch of variable intensity presented continuously upon an illuminated background, obtained very low values of ΔI—approximately 0·7 p.c. of I—at a background illumination of 3 equiv. f.c. He did not employ illuminations higher than 10 equiv. f.c. and found that at this brightness ΔI had risen to approximately 1·2 p.c. of I. In the present investigation no values of ΔI so low as 0·7 p.c. of I were obtained; this may be due to the "flickering stimulus" method here employed, or to the extended scale of the instrument, which was designed to cover values of ΔI up to 200 p.c. of I, and did not permit great precision of measurement below 1 p.c. of I. On the other hand, it is possible that the large adapting field (45° in diameter) employed in the present experiment ensured more complete adaptation and so improved differential sensitivity at high illuminations.

The present findings confirm those of Wright, in showing that the differential threshold is by no means proportional to the adapting intensity, and is not dependent solely upon it; on the other hand, by extending the range of illuminations beyond those used by him, a very marked rise has been found where the eye is adapted to brightnesses far different from those at which brightness discrimination is measured. Mellone & Rawdon-Smith [1935, unpublished], using a fairly narrow range of intensities, found an effect of this kind, and concluded that the differential threshold is lowest where the adapting and testing illuminations are equal. The present results confirm this over the range employed by them, though it does not hold for illuminations below 10^{-3} equiv. f.c. The differential sensitivity here is lower with equal "test" and "adapt" illuminations than it is if the test somewhat exceeds the adapting illumination. This is in accordance with the well-known rise of the $\Delta I/I$ curve at low intensities, showing failure of "Weber's law". The eye is much more sensitive at illuminations above 0·1 equiv. f.c., and this more than compensates for the decrease in sensitivity occasioned by inadequate adaptation to these higher illuminations, when the eye has been just previously dark adapted.

An examination of the curves for the four highest illuminations, for central vision (and for "mixed" vision, disregarding the rise at test

intensities above the adaptation illumination), suggests that one typical curve could be made roughly to fit them all, provided that the "equal test and adapt" points were made to coincide for each curve in turn. Thus, the absolute threshold is always approximately 1/5000th of the adapting illumination [cf. Blanchard, 1918; Nutting, 1920; Noldt, 1925; Wright, 1937]; the differential threshold is always about 100 p.c. at 1/1000 of the adapting intensity, and so on. Thus the shift of the curves along the abscissæ is proportional, within the limits of error, to the change in adapting illumination.

A tentative explanation of these effects may be suggested on the following lines. Under any particular adapting illumination from 4 equiv. f.c. to at least 4000 equiv. f.c. the eye possesses some degree of differential sensitivity from 1/1000 of the adapting illumination, up to and beyond the adapting illumination. Over the limited number of higher illuminations at which the differential threshold could be measured, a slight decline in differential sensitivity was again found when the test illumination was 1000 or 10,000 times as great as the adapting illumination. Over the middle third of this range, when "test" and "adapt" illuminations are nearly equal, the differential sensitivity of the eye is very acute. Adaptation to different illuminations may shift this range bodily, by an amount corresponding to the change in adapting illumination, over a range of at least 1000 : 1, thus extending the eye's instantaneous range of sensitivity a thousandfold.

We may take the analogy, employed by Lythgoe [1936], of an ammeter having various shunts. This will act as a multi-range meter giving full-scale deflexion with different currents, according to the shunt employed. At any one value of the shunt, the instrument can indicate differences in current over the whole of its scale; a set of such readings, from zero to nearly full-scale deflexion, would be roughly equivalent to one of the present curves (Figs. 3, 4), giving the differential threshold under a constant adapting illumination, at various intensities from the absolute threshold (under those conditions) up to dazzlingly bright illuminations. Changing the adapting illumination is equivalent to changing the shunt, so giving the meter a different range. Further, the fact that the differential threshold is, in general, lowest when "test" and "adapt" illuminations are equal, shows that, on this interpretation, the eye, after continued exposure to a given illumination, automatically sets itself to the range which gives greatest sensitivity at that intensity.

Thus the differential threshold of the eye appears to be a function of two variables: (1) the adapting illumination, which determines the range

over which the eye shows differential sensitivity, and (2) the test illumination, to which it gives a certain response, just as the needle of an ammeter which is set to a particular range, takes up a certain position in response to a certain current. Early methods of studying brightness discrimination appear to have hidden this dual mechanism, for there was sufficient time for the eye to adapt itself partially to each test illumination, and to set itself to the optimal range for that intensity. In normal perception, on the other hand, the eye does not have time to adapt itself to the illumination of every object it observes; it presumably attains a certain average level of adaptation, which drifts slowly with changing general conditions of illumination. Its power of discrimination at other illuminations, differing more or less from that to which it is adapted, is probably important in normal perception.

Summary

The sensitivity of the eye to differences in brightness has been investigated after adaptation to a wide range of intensities greater, equal to, and less than, those at which it was tested.

The differential threshold is, in general, found to be lowest—approximately 1 p.c.—where the "test" and "adapt" illuminations are equal; it is raised very considerably, e.g. to 100 p.c., by adaptation to illuminations greatly exceeding the test intensity. Adaptation to illuminations far below the test intensity has a similar, though less marked effect, which is greater when using a large test field than with a small centrally fixated one.

A tentative interpretation of these results is given.

In conclusion, I wish to record my deep indebtedness to Dr A. F. Rawdon-Smith, who proposed the experiment and has given invaluable advice and assistance throughout.

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ACTION OF HORDENINE COMPOUNDS ON THE CENTRAL NERVOUS SYSTEM

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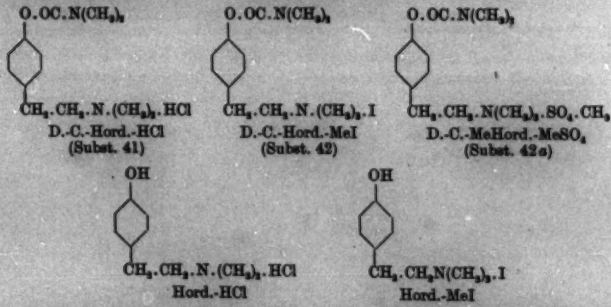
(*Received* 3 *February* 1938)

In continuation of our previous studies [Schweitzer & Wright, 1937*b*, *c*, *d*; Kremer *et al.* 1937] of the action of anticholinesterases and of acetylcholine and related substances on spinal reflexes we have examined the effects of dimethylcarbamic ester of hordenine hydrochloride (or sulphate), dimethylcarbamic ester of hordenine methiodide (or methylsulphate), dimethylcarbamic ester of methyl hordenine methylsulphate, hordenine hydrochloride (or sulphate) and hordenine methiodide (or methylsulphate). A short preliminary report has already appeared, [Schweitzer and Wright 1938]. The chemical relationships of these substances are indicated in the following formulæ. The abbreviations that will be employed when referring to these compounds are also set out. The numbers in brackets refer to the series of anticholinesterases studied by Aeschlimann & Reinert [1931]:



D.-C.-Hord.-HCl (or SO_4) and D.-C.-Hord.-MeI (or $MeSO_4$) are of special interest because they have an almost identical anticholinesterase

activity *in vitro* [Stedman, 1937]. The anticholinesterase activity of these substances is due to the urethane (dimethylcarbamic) grouping. The study of the action of Hord.-HCl or Hord.-MeI would therefore throw some light on the degree to which the effects of the dimethylcarbamic compounds were dependent on their anticholinesterase activity.

The general pharmacological activity of the hordenine base was recently studied by Rietschel [1937], and extensive investigations of the effects of hordenine sulphate were carried out by L. Camus [1906].

Methods

Cats were used under chloralose anæsthesia (0·065–0·08 g./kg. body weight). Both hindlimbs were firmly fixed by means of drills through the femur and the ischial tuberosity. The right knee jerk was usually recorded as follows. The limb was flexed at the knee joint and the patellar tendon was regularly tapped at intervals of 10 sec. with an electrically operated automatic hammer [Schweitzer & Wright, 1937*a*]. The extension movements of the limb were recorded with a torsion lever myograph. In the opposite limb the response of the gastrocnemius muscle to submaximal or maximal stimulation of its motor nerve was recorded with another torsion lever myograph. The nerve was stimulated with a fluid electrode of the type described by Collison [1933] at the same rate as the knee jerk was elicited. If the patellar reflex was feeble because of the depth of anæsthesia or for other reasons, strychnine in doses of 0·05–0·1 mg./kg. body weight was injected intravenously to raise the reflex excitability of the spinal cord.

To determine the extent to which the drugs under examination produced their effects by a peripheral action on the muscle or by an effect on the central nervous system, we used the ischæmic hindlimb technique previously described [Schweitzer & Wright, 1937*c*, *d*]. The abdominal aorta and the inferior vena cava were exposed, and all branches of the aorta below the diaphragm with the exception of both common iliacs were carefully cut between ligatures. The internal mammary arteries were tied on both sides in the second intercostal space. We have previously established that obstruction of the abdominal aorta and inferior vena cava under these experimental conditions prevents any significant amount of blood from reaching the hindlimbs. A control period of ischæmia of 5–10 min. usually showed that the force of contraction of the knee jerk and of the gastrocnemius of the opposite side was little changed. The clamps were then removed from the vessels and time was allowed for recovery. The aorta and inferior vena cava were clamped again and the

drug injected into the jugular vein. Any effects produced by such an injection during the period of ischæmia cannot be attributed to a peripheral action of the substance on the muscle but must be due to an effect on the central nervous system.

We have very frequently observed on releasing the clamps after a period of ischæmia of 5–10 min. duration that a marked fall of blood pressure occurs (Figs. 7, 9, 11) from which occasionally no recovery takes place. This has been noted whether drugs were injected during the period of ischæmia or not. In uninjected animals the knee jerk may decrease or disappear coincidentally with the fall of blood pressure. We have not yet analysed this phenomenon further, but possibly this fall of blood pressure may be related to the reactive hyperæmia observed by Barsoum & Gaddum [1935] under similar conditions. In view of the fact that injected histamine can depress the patellar reflex by a central action [Schweitzer & Wright, 1937*a*] the depression referred to above may also be found to be due to the release of some chemical agent from the ischæmic region.

Many of the experiments referred to below were carried out in atropinized animals. We have again observed, as already previously described [Schweitzer & Wright, 1937*b*], that atropine in doses sufficient to block the transmission of parasympathetic impulses (0·5–1·0 mg./kg. body weight) may itself depress or even abolish the knee jerk. Usually recovery to the pre-atropine level takes place, but in some cases full recovery does not occur. When animals under light anæsthesia showing spontaneous movement were given atropine they invariably became calmer and the heightened reflex excitability was depressed.

All injections were made into the jugular vein. The drugs were dissolved in acid saline at *p*H 4·0.

Action of dimethylcarbamic ester of hordenine hydrochloride (D.-C.-Hord.-HCl)

Intravenous injection of D.-C.-Hord.-HCl in unatropinized cats under chloralose anæsthesia increases the knee jerk and produces convulsions (Figs. 1, 2). The minimum dose to affect the knee jerk is about 5 mg./kg. body weight. With threshold doses the increase in the knee jerk begins after a latent period of 3 min. or longer, the maximum level is attained in 10–15 min., is kept up for about another 15 min., and then shows a very slow decline. Slight convulsive movements occur and are usually first noted at about the height of the knee jerk's increase. They may occur spontaneously, or may be elicited by striking the operating table.

With large doses (e.g. 10–30 mg./kg. body weight) the increase in the knee jerk takes place after a shorter latent period and develops to its maximum more rapidly. At the height of the response the knee jerk may be five or six times as great as it was initially. There may be an increase in resting quadriceps tone which precedes or accompanies the increase in the knee jerk. The convulsions set in sooner, are more violent and of greater

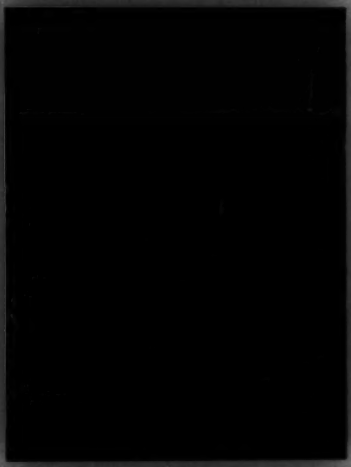


Fig. 1. Cat, chloralose. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, time in 30 sec., signal line. In all records, unless otherwise stated, the knee jerk was elicited or the nerve muscle stimulated once in 10 sec. After a control period of approx. 9 min. intrajugular injection of 50 mg. D.-C.-Hord.-HCl.

frequency, and may occur at intervals of 1 sec. or less. These points are illustrated in Figs. 1–3. Occasionally the increase in the knee jerk is preceded by an initial depression, which is always short-lived (1–2 min.), and may be slight in extent or sufficiently marked to abolish the knee jerk altogether.

Relation to atropine

As already pointed out [Schweitzer & Wright, 1937*b*] the intravenous injection of atropine in doses of 1 mg./kg. body weight usually depresses, or may even temporarily abolish the knee jerk. The subsequent

injection of D.-C.-Hord.-HCl in suitable doses increases the knee jerk and produces convulsions. These effects are little less marked than in the unatropinized animal.

Relation to blood pressure and respiration

In the doses used (5–30 mg./kg. body weight) respiration is little affected. The usual change consists of some degree of initial slowing and deepening of breathing. We have previously shown [Schweitzer & Wright, 1937*a*] that even marked alterations in pulmonary ventilation, so long as they do not produce anoxia, are practically without effect on the knee jerk.

Fig. 2. Cat, chloralose. Records from above downwards are: knee jerk (right side), blood pressure, time in 30 sec., signal line. Intrajugular injection of 20 mg. D.-C.-Hord.-HCl. Increase of quadriceps tone followed by violent convulsions.

The effect of the drug on blood pressure in the unatropinized animal is somewhat complex (Figs. 1, 2). There is an initial short, sometimes marked, fall of blood pressure followed by a fairly rapid recovery. This is usually succeeded by a second slow and progressive fall of blood pressure which varies in extent with the doses employed. Recovery takes place slowly. We have not observed any relationship between the changes in the blood pressure and in the knee jerk. We have previously shown that changes in the arterial blood pressure have little effect on reflex action unless they involve a very severe and sudden diminution in the blood supply to the central nervous system. The alterations in blood pressure which occurred in the majority of our experiments were not of a degree which, by themselves, would influence reflex activity.

In spite of the absence of any marked changes in the blood pressure of atropinized preparations D.-C.-Hord.-HCl nevertheless brings about its usual characteristic alterations in the knee jerk and in general reflex excitability.

The relation of the changes in the knee jerk to those in the nerve-muscle preparation

The doses of D.-C.-Hord.-HCl employed usually produce, on first injection, some degree of potentiation of the response of the muscle to maximal or submaximal motor nerve stimulation, at the rate of once in 10 sec. (Figs. 1, 3). This peripheral potentiation, however, can, at most, account only in part for the increase in knee jerk which has been observed. The increase in the knee jerk is always greater than that in the nerve-muscle preparation. The maximal peripheral potentiation which we have observed is twofold, commonly much less and not infrequently negligible in extent. The increase in the knee jerk, as has been pointed out, may be sixfold, and in some cases where the knee jerk has previously been absent it may return and become well developed.

Various workers, e.g. Bacq & Brown [1937] Schweitzer & Wright [1937*c*], and Briscoe [1936], have shown that the action of the anti-cholinesterases on the nerve-muscle preparation depends on the dose and on the frequency of the stimulation employed. As the frequency of the stimulation is increased a dose which previously produced peripheral potentiation may give rise to a peripheral depression of the response. In our experiments with the nerve-muscle preparation, single break shocks were employed at 10 sec. intervals. The available evidence shows that the degree of potentiation of the nerve-muscle preparation in this particular type of experiment indicates the *maximum* degree to which the drug could have enhanced the knee jerk by a purely peripheral action on the motor endings in the quadriceps muscle. When the knee jerk is increased to a much greater extent than the nerve-muscle preparation, a certain degree of stimulation of the spinal centres has probably taken place.

There is no regular time relationship between the changes in the knee jerk and in the nerve-muscle preparation. The peripheral response may return to normal while the knee jerk is still maintained at its maximum level; or, conversely, the peripheral response may remain enhanced while the patellar reflex begins to decline.

The convulsions clearly cannot be attributed to any peripheral changes. Thus, Fig. 3 shows tonic and clonic movements when the

Fig. 3. Cat, chloralose. Records from above downwards are: knee jerk (right side), contractions of gastrocnemius (left side) stimulated through its motor nerve, blood pressure, time in 30 sec., signal line. A, inject 20 mg. D.-C.-Hord.-HCl. Increase in quadriceps tone and knee jerk and onset of convulsions in the right hindlimb. B, after an interval of 10 min. C, 10 min. after record B. Faster drum. Each *X* on knee jerk record indicates response to patellar stimulation; the other movements are spontaneous.

response of the nerve-muscle preparation had returned to normal. These convulsions must be due, therefore, to a state of heightened excitability of the spinal cord leading either to a spontaneous discharge of the spinal motor cells or else to an excessive response to the afferent impulses which are constantly playing upon them.

Experiments on the ischæmic hindlimb

The results recorded so far show quite clearly that the changes in the knee jerk and the convulsions are due mainly to an action of the drug on the central nervous system. To prove this finally, experiments were

Fig. 4. Cat, chloralose. Records from above downwards are: knee jerk (right side), blood pressure, time in 30 sec., signal line. Abdominal aorta and inferior vena cava prepared. A, control period of ischæmia. B, first arrow, clamp aorta and vena cava. Second arrow, intrajugular injection of 25 mg. D.-C.-Hord.-HCl. Third arrow, release clamps.

carried out with the circulation to the hindlimbs occluded, using the technique we have described before [Schweitzer & Wright, 1937*c*, *d*]. The abdominal aorta and inferior vena cava were clamped and the drug injected into the jugular vein (Fig. 4). The usual increase of the knee jerk and the convulsions took place as in the intact animal, although none of the drug could have reached the periphery, as was borne out by the complete absence of twitching in the ischæmic limb, at a time when twitching was obvious in the forepart of the animal. On restoring the circulation to the hindlimb, no alteration in the reflex activity took place.

It may be concluded, therefore, that D.-C.-Hord.-HCl acts on the spinal centres, facilitating transmission and producing convulsions. All the actions of the drug are qualitatively identical with those which we

have previously described for eserine. Quantitatively, however, there is a marked difference, as doses 50–100 times greater have to be employed to produce comparable results.

Action of dimethyl carbamic ester of hordenine methiodide, or methylsulphate (D.-C.-Hord.-MeI *or* $MeSO_4$)

The methiodide and the methylsulphate of the dimethylcarbamic ester of hordenine have identical action, and the term D.-C.-Hord.-MeI will be used indiscriminately in this account. Intravenous injection of

Fig. 5. Cat, chloralose. Records from above downwards are: knee jerk (right side), contractions of gastrocnemius (left side) stimulated through its motor nerve, time in 30 sec., signal line. Blood pressure values indicated at arrows in mm. Hg. Inject 50 mg. D.-C.-Hord.-MeI.

D.-C.-Hord.-MeI in unatropinized cats under chloralose anæsthesia diminishes or abolishes the knee jerk. The minimum dose to produce this effect is about 5 mg./kg. body weight. With these doses the knee jerk gradually declines, and reaches its minimum after approximately 10 min., full recovery taking place after about 30 min. With bigger doses (10–30 mg./kg. body weight) the knee jerk is abolished after a short latent period, and may not show any sign of spontaneous recovery even after 1 hr. (Fig. 5).

Similar results are obtained in the atropinized animal.

Relation to blood pressure and respiration

The effects on blood pressure in the unatropinized animal are usually much less marked than those produced by the D.-C.-Hord.-HCl. With small doses no changes occur (Fig. 6). With big doses (e.g. 10–30 mg./kg. body weight) there may occur a sharp initial rise of blood pressure

followed by a small fall. Breathing is little affected, though there is sometimes a slight initial stimulation. Circulatory and respiratory changes therefore play no part in producing the results described.

Fig. 6. Cat, chloralose. Records from above downwards are: quantitative record of breathing (Wright's method, 1934, inspiration downwards), knee jerk (right side), contractions of gastrocnemius (left side) stimulated through its motor nerve, blood pressure, time in 30 sec., signal line. A, first arrow: inject 30 mg. D.-C.-Hord.-MeI. Second arrow: inject 40 mg. D.-C.-Hord.-HCl. B, record obtained after interval of 16 min.

Relation of changes in the knee jerk to those in the nerve-muscle preparation

The peripheral action of D.-C.-Hord.-MeI on the response of muscle to maximal or submaximal stimulation of its motor nerve is identical with that already described for D.-C.-Hord.-HCl. Thus, in Fig. 5 a considerable degree of potentiation of the nerve-muscle response is seen to occur simultaneously with a complete abolition of the knee jerk. There is again no time relationship between the two processes; in this experiment, for example, the knee jerk disappeared before the changes in the nerve-muscle preparation commenced. It was still absent when the nerve-muscle preparation had returned to its pre-injection state. This evidence, however, is not conclusive because the arguments previously used in

connexion with D.-C.-Hord.-HCl apply equally here, and suggest, that although the muscle stimulated through its motor nerve shows a potentiated response to single break shock at the rate of 1 in 10 sec., the quadriceps of the opposite limb might have been depressed in response to the short repetitive bursts which occur during the knee jerk. Examination of respiratory records (Fig. 6) shows that when the knee jerk is completely abolished by the drug, breathing may be unimpaired or even stimulated, suggesting that peripheral responsiveness of the respiratory muscles to repetitive stimulation is little affected.

Experiments on the ischæmic hindlimb

Experiments were carried out in our usual manner on the ischæmic hindlimb. Fig. 7 shows a typical result. The injection of 80 mg. D.-C.-Hord.-$MeSO_4$ into the jugular vein during a period of peripheral ischæmia

Fig. 7. Cat, chloralose. Records from above downwards are: knee jerk (right side), contractions of gastrocnemius (left side) stimulated through its motor nerve, blood pressure, time in 30 sec., signal line. Abdominal aorta and inferior vena cava prepared. First arrow: clamp abdominal aorta and inferior vena cava. Second arrow: intrajugular injection of 80 mg. D.-C.-Hord.-$MeSO_4$. Third arrow: release clamps. After opening the circulation to the hindlimb the gastrocnemius response to break shock stimulation of its motor nerve is potentiated.

depressed the knee jerk after a latent period of 1 min. and abolished it after 2 min. without modification in the state of the stimulated muscle on the opposite side. On releasing the clamps, peripheral potentiation of the nerve-muscle preparation occurred without recovery of the knee jerk.

It may be concluded, therefore, that D.-C.-Hord.-MeI depresses or abolishes the patellar reflex largely owing to an inhibitory action on the spinal cord. Its action is qualitatively identical with that of certain other anticholinesterases which we have examined, namely, prostigmine, Stedman's "Meta"-MeI compound, substances 13 and 36 of the Aeschlimann & Reinert series, and acetylcholine and certain other choline esters.

Briscoe [1938] has shown that D.-C.-Hord.-HCl and D.-C.-Hord.-MeI have the same qualitative effect, and almost the same quantitative effect on the response of the nerve-muscle preparation of the cat under dial anæsthesia when stimulated electrically at rates varying from 30 to 150 per sec. That these drugs have identical action on skeletal muscle, both when very slow and very high rates of stimulation of the motor nerve are employed, proves conclusively that the difference in the action of the two substances on spinal reflexes must be due to differences in their action on the central nervous system.

Mutual antagonism between D.-C.-Hord.-HCl *and* D.-C.-Hord.-MeI

The intravenous injection of D.-C.-Hord.-HCl in suitable doses annuls the inhibitory effect on the patellar reflex of a previous injection of D.-C.-Hord.-MeI. If the doses are suitably adjusted (Fig. 6), D.-C.-Hord.-HCl produces the characteristic increase in the knee jerk and changes in resting quadriceps tone and violent convulsions may occur. Conversely, the convulsant action of D.-C.-Hord.-HCl can be depressed or abolished by a suitable dose of D.-C.-Hord.-MeI. The results obtained in any particular experiment depend on the initial state of the animal and the relative doses of the two drugs employed.

Action of dimethylcarbamic ester of methyl hordenine methylsulphate (D.-C.-Me.-Hord.-$MeSO_4$)

The action of this substance is in all essential respects the same as that of D.-C.-Hord.-MeI. Intrajugular injection of D.-C.-Me.-Hord.-$MeSO_4$ in doses of 10–20 mg./kg. body weight abolishes the patellar reflex under conditions of complete anæmia of the hindlimbs.

Action of hordenine salts

Removal of the dimethylcarbamic grouping from D.-C.-Hord.-HCl and D.-C.-Hord.-MeI abolishes their anticholinesterase action *in vitro*. We have used hordenine HCl or sulphate, and hordenine methiodide or methylsulphate.

Action of hordenine HCl *and hordenine sulphate*

Both the salts have identical actions on spinal reflexes, and in the account that follows the term Hord.-HCl will be used indiscriminately for either substance.

In the unatropinized animal an intravenous injection of Hord.-HCl of 5 mg./kg. body weight produces a considerable diminution of the knee jerk with slow recovery. Large doses (10–30 mg./kg. body weight)

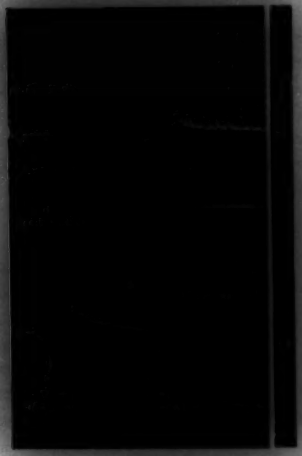


Fig. 8.

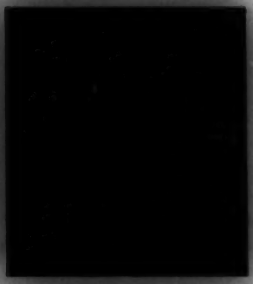


Fig. 9.

Fig. 8. Cat, chloralose. Records from above downwards are: knee jerk (right side), contractions of gastrocnemius (left side) stimulated through its motor nerve, blood pressure, time in 30 sec., signal line. A, first arrow: inject 120 mg. Hord.-HCl. Second arrow: inject 100 mg. D.-C.-Hord.-HCl. B, 75 min. after A.

Fig. 9. Cat, chloralose. Abdominal aorta and inferior vena cava prepared. Records from above downwards are: knee jerk (right side), blood pressure, time in 30 sec., signal line. First arrow: clamp abdominal aorta and inferior vena cava. Second arrow: intrajugular injection of 100 mg. Hord.-HCl. Third arrow: release clamps; violent fibrillary twitching is recorded in the limb. Note marked after-fall of blood pressure and slow recovery.

abolish the knee jerk, which may show no sign of recovery even after 30 min. (Fig. 8). Smaller doses have no action on the response of the nerve-muscle preparation to single break shocks at intervals of 1 in 10 sec. With the larger doses (30 mg./kg.) there is a small transient

depression in the peripheral responses, but the patellar reflex remains absent for a long time after the peripheral preparation has returned to its pre-injection state (Fig. 8).

The injection of D.-C.-Hord.-HCl following the inhibition produced by Hord.-HCl leads to the usual increase in resting quadriceps tone and in the knee jerk, and subsequently to the appearance of convulsions (Fig. 8).

Hordenine HCl in the unatropinized animal produces a marked effect on blood pressure. There may be an initial fall followed by a rise and subsequent gradual return to normal. The changes in the knee jerk are entirely unrelated to those in the circulation. Respiration is not depressed and marked initial stimulation may occur, while the knee jerk is absent.

Using our usual ischæmic technique, it can be shown that, after clamping the abdominal aorta and inferior vena cava, the injection of Hord.-HCl in suitable doses into the jugular vein will also abolish the knee jerk (Fig. 9). In this experiment, after releasing the clamps, violent twitching, which had previously taken place in the forepart of the animal, also developes in the hindlimbs. The substance, therefore, exercises a depressant action on the spinal cord.

Action of hordenine methiodide or methylsulphate
(Hord.-MeI *or* $MeSO_4$)

These substances are far more potent than the corresponding HCl or sulphate. Intravenous injection of Hord.-$MeSO_4$ in doses of 0·3–0·5 mg./kg. body weight abolishes the knee jerk, produces violent twitching of the skeletal muscles, arrests respiration, and depresses the response of skeletal muscle to maximal or submaximal stimulation.

The abolition of the knee jerk long outlasts the peripheral depressant action on the nerve-muscle preparation (Fig. 10); with larger doses well-marked contracture may occur. Similar results are obtained if artificial respiration is maintained throughout the experiment and if the animal is atropinized (1 mg./kg. body weight). These substances also produce blood pressure changes like those resulting from Hord.-HCl, but unrelated to the changes in the knee jerk.

As a quarternary ammonium derivative ["onium salt", Ing, 1936] Hord.-$MeSO_4$ has a curarizing action in accordance with expectation. It was obviously very important to carry out stringent tests to determine whether the observed abolition of the patellar reflex was due in any degree to the action of the drug on the central nervous system, or was wholly peripheral in origin. Fig. 11 illustrates an experiment carried out during rigidly controlled ischæmia of the hindlimb, and shows that

under these conditions also, intrajugular injection of Hord.-$MeSO_4$ in suitable doses (2 mg.) abolished the knee jerk, artificial respiration being maintained throughout the experiment.

It can be concluded that Hord.-$MeSO_4$ or Hord.-MeI has a central depressant action which is about 50 times as great as that of the corresponding D.-C.-Hord.-MeI.

Fig. 10.

Fig. 11.

Fig. 10. Cat, chloralose. Records from above downwards are: knee jerk (right side), contractions of gastrocnemius (left side), stimulated through its motor nerve, blood pressure, time in 30 sec., signal line. A, inject 1 mg. Hord.-MeI intravenously. Knee jerk abolished, nerve-muscle preparation temporarily depressed. B, 20 min. after A.

Fig. 11. Cat, chloralose. Abdominal aorta and inferior vena cava prepared. Records from above downwards are: knee jerk (right side), contractions of gastrocnemius (left side) stimulated through its motor nerve. First arrow: clamp abdominal aorta and inferior vena cava. Second arrow: intrajugular injection 2 mg. Hord.-MeI. Third arrow: release clamps. Note after-fall of blood pressure.

Discussion

D.-C.-Hord.-HCl and D.-C.-Hord.MeI have an identical anticholinesterase action *in vitro*, and produce effects, which are identical qualitatively and very similar quantitatively, on the response of the nerve-muscle preparation, both at low and high rates of stimulation. D.-C.-Hord.-HCl is a convulsant and D.-C.-Hord.-MeI depresses somatic reflexes. The diametrically opposite effects which they produce on reflexes must, therefore, be due to differences in their action on the central nervous system. The changes in blood pressure and respiration play no

significant part. It can be concluded that the two drugs act directly and differently on the central nervous system, probably the spinal cord. D.-C.-Hord.-HCl acts like eserine; D.-C.-Hord.-MeI acts like prostigmine and intravenously injected acetylcholine itself.

The intensity of the convulsant action of D.-C.-Hord.-HCl is about 50–100 times less than that of eserine, and the anticholinesterase activity of the two substances bears a similar relationship. Similarly, the depressant action of D.-C.-Hord.-MeI is related to its anticholinesterase activity; both of these properties are much weaker in the case of D.-C.-Hord.-MeI than for prostigmine or Stedman's meta-methiodide compound.

The intensity of action of both D.-C.-Hord. compounds is thus related to their anticholinesterase action, but the qualitative nature of the effect, i.e. whether they will produce central excitation or central inhibition, obviously cannot be explained simply on these grounds.

Removal of the anticholinesterase grouping from D.-C.-Hord.-HCl abolishes its convulsant action and, as we have shown, Hord.-HCl is a central depressant. Similar treatment of D.-C.-Hord.-MeI to give rise to Hord.-MeI greatly intensifies its depressant action. The presence of the anticholinesterase grouping therefore significantly influences the mode of action of these substances. We propose to defer fuller discussion of the implications of these results to our next paper. At this stage we only wish to comment further on one point. Hitherto eserine was the only convulsant anticholinesterase known. The other anticholinesterases examined and intravenously injected acetylcholine itself are all central depressants. We were therefore led to suggest that the central excitatory action of eserine was *not* due to its anticholinesterase activity, but to some other chemical property of the molecule. In the light of the evidence presented above, this conclusion must be modified. In D.-C.-Hord.-HCl we have an example of another anticholinesterase with convulsant properties. We have shown, further, that the convulsant action disappears (and is, in fact, replaced by a depressant action) when the dimethylcarbamic grouping is removed, thus depriving the substance of its anticholinesterase activity. It seems probable, therefore, that anticholinesterases, *by virtue of being anticholinesterases, may be central depressants or convulsants.* We will consider possible explanations of this phenomenon in our next paper.

SUMMARY

1. The central action in cats under chloralose anæsthesia of dimethylcarbamic ester of hordenine HCl, dimethylcarbamic ester of hordenine methiodide, dimethylcarbamic ester of methyl hordenine methylsulphate, hordenine HCl and hordenine methiodide or methylsulphate was examined.

2. D.-C.-Hord.-HCl and D.-C.-Hord.-MeI have similar anticholinesterase action *in vitro*, and similar actions qualitatively and quantitatively on the response of muscle to motor-nerve stimulation.

3. D.-C.-Hord.-HCl increases muscle tone and the knee jerk and produces convulsions, mainly by a stimulating action on the spinal cord. Its action is qualitatively identical with that of eserine, but quantitatively it is 50–100 times more feeble.

4. D.-C.-Hord.-MeI and D.-C.-MeHord.-$MeSO_4$ depress the spinal cord and diminish or abolish the knee jerk. Qualitatively this action is identical with that of prostigmine and certain other anticholinesterases previously described.

5. Hord.-HCl is a central depressant. Removal of the anticholinesterase grouping from D.-C.-Hord.-HCl thus abolishes its central convulsant action.

6. Hord.-MeI is a central depressant, about 50–100 times more potent than D.-C.-Hord.-MeI.

7. The theoretical implications of these results are briefly discussed.

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THE RESPIRATION AND METABOLISM OF SUBMAXILLARY GLAND TISSUE OF THE CAT

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It has been shown previously [Deutsch & Raper, 1936] that the respiration of thin slices of salivary gland tissue is increased by various chemical agents such as acetylcholine, adrenaline and eserine which cause secretion in the intact gland in the living animal. It was shown further that this increase of respiration is inhibited by atropine in those instances in which atropine inhibits secretion *in vivo*. In addition, it was found that the magnitude of the increased respiration shown by the tissue slices *in vitro* is similar to that observed by earlier investigators when the intact gland is stimulated through its nerve.

This close parallelism between the respiratory phenomena of slices of the gland and the gland itself *in vivo* indicated that the method might be useful for studying the metabolism accompanying activity.

It has to be borne in mind, however, that although secretory activity is coupled with an increased oxygen consumption, the converse that oxygen uptake is invariably accompanied by secretory activity, is probably not true. This may sometimes mean a limitation in the usefulness of the method, but the great advantage remains that it has considerable adaptability and yields information which could not be obtained by the *in vivo* method.

In this paper a number of observations are recorded of the respiration of slices of the cat's submaxillary gland under a variety of conditions. They were made with the object of elucidating the relationship between secretory activity and respiration, and also with the idea that they would yield information about the metabolic processes accompanying activity. The cat's submaxillary gland was chosen because of the large effects which accompany the application of stimulating agents.

Methods

In order to reduce variations which must occur because tissue slices of the same gland do not all possess exactly the same percentage of secretory tissue, consecutive slices were cut from the same gland using the method previously described by Deutsch [1936]. When the cross-section of the gland exposed the somewhat tougher tissue of the system of the major ducts near the hilus, this tissue, which is marked by its whitish colour, was removed before the next section was taken. In addition, where a great number of manometers was used in one comparative experiment, the experiments were repeated a sufficient number of times to exclude errors caused by naturally occurring variations between individual slices. The manometric measurements were carried out according to Warburg's [1930] method with manometers having cone-shaped flasks with side bulb and inner container or with flasks of rectangular shape with side bulb but without inner container. In a special series of experiments manometers of the Dixon-Keilin [1933] type were used. A mixture of acetylcholine bromide and eserine sulphate (denoted in this paper as ACh.) in various concentrations was used for stimulating the tissue to activity. It was placed in the side bulb of the Warburg flasks and tipped into the main vessel. A period of 2 min. proved sufficient after the addition to ensure compensation of the resulting disturbance. When the Dixon-Keilin apparatus was used the ACh. was placed in a small cup hung from the inner container and was dislodged when required in the usual way. The temperature in all experiments was 37·5° C. The gas space in the flasks was filled with oxygen when the oxygen uptake only was measured, and with oxygen and 5 p.c. carbon dioxide when the carbon-dioxide output was being estimated at the same time. For the anaerobic experiments a mixture of nitrogen and 5 p.c. carbon dioxide was used. Traces of oxygen were removed from the nitrogen by the use of a hot copper spiral. The Ringer's solution contained 3×10^{-3} *M* $NaHCO_3$ and 0·2 p.c. glucose (if not otherwise stated) for the estimation of O_2 uptake only, and $2{\cdot}5 \times 10^{-2}$ *M* $NaHCO_3$ when the CO_2 output was also being measured.

The symbols used in this paper to express the metabolism are:

$$Q_{O_2} = \frac{\mu\text{l. } O_2 \text{ consumed in } O_2}{\text{mg. tissue (dry wt.)} \times \text{hr.}};$$

$$Q_S^{O_2} = \frac{\mu\text{l. acid (carbonic and lactic) formed in } O_2}{\text{mg. tissue (dry wt.)} \times \text{hr.}};$$

$$Q_M^{O_2} = \frac{\mu\text{l. lactic acid formed in } O_2}{\text{mg. tissue (dry wt.)} \times \text{hr.}};$$

$$Q_M^{N_2} = \frac{\mu\text{l. lactic acid formed in } N_2}{\text{mg. tissue (dry wt.)} \times \text{hr.}}.$$

Before removing the glands the animals were anæsthetized with ether. It is important that the anæsthesia should be of the shortest possible duration, and this was ensured by hanging a ball of cotton wool soaked with ether at the *top* of the bell jar covering the cat.

Results

Metabolic effects following stimulation

When the respiration of gland slices was studied without using KOH for the absorption of CO_2, i.e. in oxygen and 5 p.c. CO_2, the addition of ACh. caused a development of positive pressure during the first 10 min. Details of an experiment of this kind are recorded in Table I in order to show the course of the reaction as time goes on. In manometer 1 the tissue was stimulated aerobically, and in manometer 2 anaerobically. Several slices were used in both manometers. Manometer 3, without tissue, served as a control and showed whether the addition of ACh. itself caused any manometric change.

Table I. Early manometric changes produced by the addition of ACh. Concentration in Ringer's solution: glucose, 0·2 p.c., $NaHCO_3$ $2·5 \times 10^{-3}$ *M*. (ns) = non-stop reading. Manometric readings in mm. The figures outside the brackets represent mm. change in the corresponding time corrected for thermobarometric change.

Time	Thermo-barometer Air	Manometer 1 O_2 + 5 p.c. CO_2. Eserine, 1/234,000; acetylcholine, 1×10^{-4}		Manometer 2 N_2 + 5 p.c. CO_2		Manometer 3 O_2 + 5 p.c. CO_2. Control without tissue. Eserine, 1/234,000; acetylcholine, 1×10^{-4}
11·15	63·5	51·0		−45·0		57·0
			−11·5		+17·0	
11·25	64·5	41·0	−11·5	−27·0	+14·5	58·5
11·35	65·0	30·0		−12·0		59·0
11·36	—	Added ACh.	+2·0	—		Added ACh.
11·39	66·0	33·0		− 5·0		60·0
11·41 (ns)	67·0	40·0		—		61·5
11·42	67·0	42·5		—		61·5
11·44	67·0	44·5	+13·0	+ 3·0	+14·5	61·0
11·46 (ns)	67·0	47·0		—		62·0
11·49	66·0	46·0		+11·5		61·0
11·54 (ns)	68·0	47·0	−4·0	—		63·0
11·59	70·0	46·0		+31·0		65·0
			−6·0		+15·0	
12·09	71·0	41·0		+46·0		66·0
			−9·0		+14·5	
12·19	71·0	32·0		+60·5		66·0
			−7·0		+14·5	
12·29	72·0	26·0		+76·0		67·5
			−9·5		+13·5	
12·40	72·5	17·0		+90·0		—
	Dry wt. mg.	18·2		11·8		

During the period of 20 min. before adding the ACh. a negative change of 23 mm. takes place in manometer 1. At 11·39, 3 min. after adding the ACh., a positive pressure change is indicated. From this point, which serves as a new zero point after adding ACh., the positive

pressure increases till 11·44 and more slowly from 11·44 to 11·49. From 11·49 onwards the pressure change again becomes negative and remains so. The anaerobic glycolysis in manometer 2 proceeds at the average constant rate of 14·8 mm. per 10 min., which corresponds to $Q^{N_2}_{\Delta} = 9{\cdot}33$. The control manometer 3 does not change, so that the addition of ACh. from the side bulb does not interfere. This experiment shows that during the first few minutes after the addition of ACh., "extra" CO_2 is liberated, but the effect does not persist for long. That this effect is due to the action of the ACh. on the glandular tissue is shown in Table II, which gives

TABLE II. Comparison of the effect of ACh. alone and in the presence of atropine (1/3000). Other details as in Table I

Time	Thermo-barometer	Manometer 1 ACh. + atropine		Manometer 2 ACh.		Manometer 3 Atropine	
3·15	31·0	74·0		84·5		51·0	
			−4·0		−4·5		−1·0
3·25	32·0	71·0		81·0		51·0	
3·26½	—	ACh. + atropine added		ACh. added		Atropine added	
3·29	33·0	71·0		82·5		52·0	
3·31 (ns)	32·5	69·0		85·0		52·0	
			−2·0		+4·5		−1·0
3·32 (ns)	33·0	—		86·0		—	
3·34	33·0	69·0		87·0		51·0	
3·41 (ns)	31·5	64·0	−5·0	86·0	0	49·0	−1·0
3·44	33·0	64·0		87·0		50·0	
			−4·5		−2·5		−1·5
3·54	32·0	58·5		83·5		47·5	
			−4·5		−4·5		−2·0
4·04	31·0	53·5		78·5		45·0	
			−14·5		−21·0		−5·0
4·45	27·5	34·5		53·5		36·0	
			−2·5		−3·0		−1·0
4·55	27·0	31·5		50·0		34·5	
	Dry wt. mg.	12·0		9·8		5·2	

details of an experiment in which atropine was added (concentration after addition 1/3000) along with the ACh. (manometer 1). Manometer 2 served as a control and was identical with manometer 1 of Table I. Manometer 3 contained atropine alone in the side bulb. It will be seen that there was no development of positive pressure in manometer 1.

Table III shows the result when adrenaline was used instead of ACh. to stimulate the gland. The same evolution of "extra" CO_2 is observed as with ACh. The results of these three experiments are represented schematically in Fig. 1.

Other experiments in which atropine as well as adrenaline was present showed that the adrenaline effect was not abolished by atropine, so that adrenaline does not act on the gland by liberating acetylcholine.

The most obvious conclusion from these experiments would appear to be that stimulation with acetylcholine or adrenaline gives rise to an aerobic glycolysis which gradually disappears, the "extra" CO_2 being liberated from bicarbonate by the lactic acid produced. To get more

TABLE III. Manometric changes produced by addition of adrenaline. The adrenaline was stabilized in the side bulb by dissolving in Ringer's solution without $NaHCO_3$ and containing HCl, $3{\cdot}1 \times 10^{-4}$ *M*. Concentration of adrenaline in main vessel after tipping, 1/480,000. Other details as in Table I.

Time	Thermo-barometer	Manometer 1 Adrenaline with tissue		Manometer 2 Adrenaline without tissue	
11·25	29·0	15·0		39·0	
11·32	31·0	13·0		41·5	
11·33½	—	Adrenaline added		Adrenaline added	
11·36	34·0	17·5		45·0	
11·38	33·0	19·0		44·0	
11·39	34·0	21·0		45·0	−1·0
11·41	34·0	22·0	+5·5	44·0	
11·44	35·5	24·0		45·5	
11·49	36·0	25·0		46·0	
11·59	37·0	21·0	−5·0	47·0	
12·09	37·0	15·5	−5·5	47·0	
12·19	38·0	11·0	−5·5	48·0	
	Dry wt. mg.	16·1			

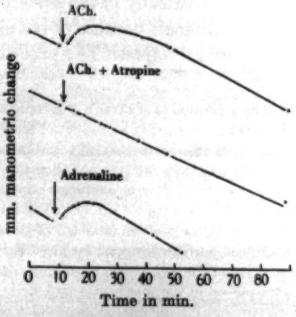

Fig. 1. Manometric changes following the addition of (*a*) ACh. with and without atropine, and (*b*) adrenaline.

quantitative information concerning this phenomenon, experiments were carried out by Warburg's differential method in two manometers, the one having a small and the other a large amount of fluid in it. Great care was taken to have identical slices in the two manometers. The fluid used was Ringer's solution with 0·2 p.c. glucose and $2{\cdot}5 \times 10^{-2}$ *M* $NaHCO_3$. *Q* values calculated from the readings in an experiment of this kind are given in Table IV.

It will be seen from Table IV that the resting tissue does not give off "extra" CO_2. During the first 5 min. after ACh. is added, "extra" CO_2 appears and the $Q_M^{O_2} = 14{\cdot}5$. The rate of development slows down quickly,

TABLE IV. Comparison of Q values before and after stimulation with ACh. Ringer's solution as in Table I

Q_{O_2}	$Q_S^{O_2}$	$Q_M^{O_2}$	State of tissue	Period
− 7·95	+ 6·5	− 1·45	Resting	10 min.
− 60·50	+ 75·0	+ 14·50	Stimulated	5 „ after adding ACh.
− 38·00	+ 44·3	+ 6·24	Stimulated	10 „ „ „
− 34·30	+ 37·9	+ 3·60	Stimulated	10 „ following
− 24·60	+ 23·7	− 0·90	Stimulated	10 „ „
− 25·20	+ 24·4	− 0·80	Stimulated	10 „ „

and the value 6·24 is obtained during the first 10 min. after tipping. During the next 10 min. the value sinks to 3·6 to be negative from then on, that is to say, more oxygen is now being used than CO_2 developed, just as during the resting period.

If the "extra" CO_2 developed in these experiments is due to formation of lactic acid as a result of stimulation, it should be possible to determine the amount by chemical methods and compare this with the amount of "extra" CO_2 measured manometrically in the same period. The chemical determination is technically difficult because of the short duration of the phenomenon, but it has been attempted. The procedure adopted was as follows:

Lactic acid was estimated by a method of Mawson & Ritchie [1932] after the removal of sugar according to Salkowski & van Slyke [1929]. The slices for the manometric determination were cut and the manometers made ready and put into the thermostat. The remaining bulk of the two glands was then cut into fine slices for the chemical determination. The slices were first rinsed in 200 ml. Ringer's solution containing 0·2 p.c. glucose and $2·5 \times 10^{-3}$ M $NaHCO_3$, saturated with oxygen + 5 p.c. CO_2. The fluid was decanted, and the slices divided into two parts as equal as possible (about 0·75 g. each). Adhering fluid was removed with filter paper, and the portions dropped into two Erlenmeyer flasks each containing 15 ml. of Ringer's solution of the composition just mentioned. After filling with the gas mixture these flasks were placed in the thermostat and shaken for 10 min. along with the manometers from which readings for the resting respiration were taken. The ACh. was then tipped into the manometers, and simultaneously an equivalent amount of ACh. was added to one of the Erlenmeyer flasks. The other flask, without ACh., served as a control. The development of positive pressure in the manometer with the smaller amount of fluid was carefully observed. When the positive pressure was judged to be at its peak value (usually after 5–7 min.), the Erlenmeyer flasks were quickly removed and the contents emptied into small Hirsch funnels which rested on centrifuge tubes containing the lime-copper mixture (2 ml. lime + 1 ml. copper solution). The time of removal was noted. The fluid ran through the funnels quickly, and any further reaction was stopped at once on contact with the lime-copper mixture. The same amount of ACh. was added to the fluid from the non-stimulated tissue in order to have identical conditions for the chemical estimation. The tissue remaining in the funnels was well rinsed, dried and weighed. The copper-lime treatment removed all traces of mucin as well as sugar and, after centrifuging, an entirely transparent fluid was obtained. After neutralization with $3N$ H_2SO_4 it was analysed for lactic acid. The calculation of the results has to take into account the various additions of fluid (ACh.; $CuSO_4$; $Ca(OH)_2$; H_2SO_4). The 2 ml. $Ca(OH)_2$ were calculated as 1 ml. because of the solid phase of the $Ca(OH)_2$.

The following are the results of two such experiments:

(1) Flasks removed 7 min. after adding ACh. $Q^{O_2}_M$ in this period = 10·7 (manometric). Chemical determination of lactic acid corresponded to $Q^{O_2}_M$ value of 6·15.

(2) Flasks removed 6 min. after adding ACh. $Q^{O_2}_M$ in this period = 16·2 (manometric). Chemical determination gave lactic acid corresponding to a $Q^{O_2}_M$ value of 6·4.

In other experiments in which the chemical determination alone was carried out $Q^{O_2}_M$ values of 6·17 and 5·6 were obtained.

These experiments show that lactic acid is produced during the period of "extra" CO_2 formation, but the amount of lactic acid estimated chemically accounts for only about half the "extra" CO_2. It must be remembered that the chemical determination is dealing with amounts which are near the lower limit of the method, and the assumption has to be made that equilibrium is attained between the tissue slices and the surrounding fluid as regards lactic-acid content. This may account in part for the deficit in lactic acid found chemically, but the possibility remains that some other acid may be produced besides lactic. Since pyruvic acid might appear as an intermediate product of the breakdown of glucose and be a source of the "extra" CO_2, the effect of sodium pyruvate as a substrate instead of glucose was examined. The pyruvic acid was redistilled, neutralized with NaHCO and used in 0·02 *M* concentration in Ringer's solution. It was found that the development of positive pressure was considerably suppressed or did not appear at all. In other experiments when fructose (0·2 p.c.) was used as substrate or when no substrate was added to the Ringer's solution, there was no production of "extra" CO_2. Data from typical experiments are given in Table V.

These experiments show that the outburst of "extra" CO_2 occurred only in presence of glucose. This fact would favour the view that there is a sudden aerobic glycolysis caused by a temporary insufficiency of the Pasteur reaction, so that the oxidation mechanism, as it were, could not cope with the sudden and extraordinary demand for oxygen. The deficit in lactic acid when estimated chemically must stand against this as a complete explanation. The deficit may be even greater than that recorded above, because 2–3 min. are allowed in the manometric measurements for the attainment of equilibrium after adding ACh., whereas during this period any lactic acid produced would be estimated by the chemical method.

Other attempts to find an explanation of the deficit were unsuccessful. The development of ammonia by deamination was considered, but no

TABLE V. *Q* values obtained by stimulating with ACh. when no substrate was added and in presence of pyruvate and fructose. The values obtained with glucose as substrate in the first two experiments are given for comparison.

Substrate	Q_{O_2}	$Q_N^{O_2}$	$Q_M^{O_2}$	State of tissue	Period	With glucose Q_{O_2}	With glucose $Q_N^{O_2}$	With glucose $Q_M^{O_2}$
Pyruvate	− 6·9	+ 2·4	− 4·5	Resting	30 min.			
	−21·7	+17·4	− 4·3	Stimulated	5 „ after adding ACh.	−50·2	+64·8	+14·6
	−19·2	+17·3	− 1·9	„	5 „ following	−40·5	+45·7	+ 5·2
	−19·1	+10·3	− 8·8	„	10 „ „	−23·6	+19·8	− 5·6
	−14·2	+10·6	− 3·6	„	10 „ „	—	—	—
	−14·2	+ 9·6	− 4·6	„	10 „ „	− 7·7	+10·6	− 2·9
None added	− 6·6	+ 5·5	− 1·1	Resting	15 „	− 6·1	+ 5·4	− 0·7
	−21·5	+21·8	+ 0·3	Stimulated	5 „ after adding ACh.	−21·6	+31·8	+10·2
	−20·8	+19·6	− 1·2	„	6 „ following	−18·7	+24·4	+ 5·7
	−20·0	+17·9	− 2·1	„	10 „ „	−21·4	+27·4	+ 6·0
	−18·8	+15·5	− 3·3	„	10 „ „	−13·8	+14·3	+ 0·5
	−18·1	+15·3	− 2·8	„	10 „ „	−13·0	+13·2	+ 0·2
Fructose	− 8·9	+ 7·6	− 1·3	Resting	20 „	—	—	—
	−19·0	+18·9	− 0·1	Stimulated	5 „ after adding ACh.	—	—	—
	−20·8	+19·1	− 1·1	„	5 „ following	—	—	—
	−16·1	+12·1	− 4·0	„	5 „ „	—	—	—

change in the ammonia content was detectable. A few experiments carried out to study change in phosphoric acid content were fruitless because the mucin interferes with the colorimetric estimation.

One further experiment may be mentioned in which the effect of ethyl carbylamine was examined. Warburg [1930] has found that this substance interrupts the Pasteur reaction and causes an aerobic glycolysis. So far as we are aware its action on the submaxillary gland has not been investigated. The results shown in Table VI, in which a 10^{-3} *M*

TABLE VI. Effect of ethylcarbylamine on the *Q* values obtained after stimulation with ACh. in presence of glucose

Q_{O_2}	$Q_S^{O_2}$	$Q_M^{O_2}$	State of tissue	Period
−20·9	+20·95	—	Resting	10 min.
−31·2	+45·00	+13·8	Stimulated	5 „ after adding ACh.
−22·9	+34·40	+11·5	Stimulated	10 „ „ „
−25·0	+30·20	+ 5·2	Stimulated	10 „ following
−25·7	+30·20	+ 4·6	Stimulated	10 „ „
−31·2	+35·90	+ 4·7	Stimulated	10 „ „

concentration was used, demonstrate that the resting tissue does not show any aerobic glycolysis in contrast to other tissues (liver, kidney, testis, embryo, Jensen's sarcoma) examined by Warburg [1930]. It is true that the resting respiration is somewhat large, but not greater than may be found under ordinary circumstances. When ACh. is added the outburst of "extra" CO_2 is extended, being still noticeable 40 min. later. The tendency of the $Q_M^{O_2}$ values to return to their value at rest is, however, just as obvious as in other experiments without ethyl carbylamine.

Anaerobic experiments

To obtain further information about the utilization of carbohydrate by the gland, a comparison has been made of the anaerobic glycolysis of the tissue at rest and stimulated by ACh. in Ringer's solution, (*a*) without substrate, (*b*) with 0·2 p.c. glucose, and (*c*) with 0·2 p.c. fructose. The results are recorded in Table VII. The resting gland shows an anaerobic

TABLE VII. Effect of ACh. under anaerobic conditions without added substrate and in presence of glucose and fructose. The figures are the $Q_M^{N_2}$ values. The rate was constant in each case.

Substrate:	None	Glucose	Fructose	
	1·76	10·9	2·75	Before adding ACh.
	1·23	9·3	2·35	After adding ACh.

glycolysis of a moderate extent only with glucose. The value with fructose is hardly more than without substrate. The addition of ACh. does not cause any increase in the rate of glycolysis with either of the substrates or

with no substrate at all. It may be concluded, therefore, that the natural carbohydrate reserve is small, and that the gland can produce lactic acid under anaerobic conditions from glucose but not from fructose. In this respect the submaxillary gland resembles carcinomatous tissue.

The effect of monoiodoacetate and fluoride

In the first section it has already been shown that the stimulated gland is able to bring about glycolytic changes in the presence of glucose. It was therefore of interest to study the effect of iodoacetate and fluoride on the gland both in the resting and stimulated state. In an experiment of this kind the respiration of slices of the same gland was compared under the following conditions, using eight manometers. In four of the manometers the tissue was placed in:

Ringer's solution + glucose.
" " + lactate.
" " + glucose with ACh. (in side bulb).
" " + lactate with ACh. (in side bulb).

The other four manometers were similar, but contained in addition iodoacetate. Table VIII gives the results of one of several such experiments.

TABLE VIII. Effect of iodoacetate with and without ACh. on the respiration in presence of glucose and lactate. The concentration of the various substances in the Ringer's solution was as follows: glucose, 0·2 p.c.; sodium *dl*—lactate 0·2 p.c.; sodium iodoacetate 6×10^{-4} *M*. The ACh. in the side bulb was arranged to give final concentrations in the main vessel of acetylcholine bromide 1/1,000,000, and eserine sulphate 1/234,000. Carbon dioxide was absorbed in 8 p.c. KOH. The tissue slices were placed in the fluid and after they had been in contact with the iodoacetate for 35 min. (this time being required for attaching flasks, filling with gas mixture etc.) the ACh. was added from the side bulb. The zero reading was taken 3–5 min. later. The figures are the Q_{O_2} values obtained during the first and second 20 min. periods after the zero reading was taken.

		No addition	With iodoacetate	With ACh., no iodoacetate	With iodoacetate and ACh.
With glucose	1st 20 min.	9·1	7·3	35·6	9·7
	2nd 20 min.	8·3	5·2	31·2	7·9
With lactate	1st 20 min.	10·3	17·5	25·6	19·2
	2nd 20 min.	9·8	13·3	21·8	14·6

It is seen that the respiration of the resting gland is invariably slightly greater with lactate as substrate than with glucose. Brain tissue and growing cells behave similarly [Krebs, 1931], whereas with muscle [Meyerhof, 1930] the respiration with lactate is greatly increased. The explanation given by Krebs that brain tissue and growing cells behave

in this way because they are already saturated with lactate on account of their great power to produce it, is probably not valid for the submaxillary gland which does not exhibit marked glycolytic action.

The stimulating effect of ACh. is equally shown whether the substrate be glucose or lactate.

The respiration of the resting gland in presence of iodoacetate decreases when glucose is the substrate, but increases by 40–70 p.c. in the presence of lactate. This behaviour corresponds to that of brain tissue, growing cells [Krebs, 1931] and resting muscle [Meyerhof & Boyland, 1931]. It is at present unexplained.

The addition of ACh. to the tissue poisoned by iodoacetate does not lead to the typical increased oxidation which is shown by the untreated gland, and the substrate (lactate or glucose) has no influence on this effect. Since the poisoning effect of iodoacetate increases with time a modified experiment was carried out, the purpose of which was to examine the relationship between the disappearance of the stimulating effect of ACh. and the poisoning effect of iodoacetate. The results are given in Table IX.

The three manometers, G, G_1 and G_2, contained "glucose-Ringer" in the main vessel and ACh. in the side bulb. G_1 and G_2 contained, in addition, iodoacetate of the concentration $1{\cdot}2 \times 10^{-3}$ M. This was double the amount used for the experiments of Table VIII. The time which elapsed between the first contact of the tissue with iodoacetate and the zero reading was 20 min. The respiration during the first 10 min. was observed, ACh. being still in the side bulb of all three manometers. The ACh. was then tipped into the main vessel as follows: in G_1 at the 11th min., in G_2 at the 21st min. and in G at the 41st min.

TABLE IX. The effect of ACh. on the respiration after various times of contact of the tissue with iodoacetate. The figures are the Q_{O_2} values in successive 10 min. periods.

G	G_1	G_2
Without iodoacetate	With iodoacetate	
10·40	9·55	9·15
10·40	8·25 + ACh.	7·25
10·99	7·80	7·25 + ACh.
10·49	6·08	5·45
18·0 + ACh.	4·34	4·08

The figures show that the respiration during the first 10 min. is already slightly depressed by the iodoacetate. During the second 10 min. it is held near its previous value in G_1 by the ACh. added, but goes on diminishing in G_2. The further decline in G_2 is arrested for a short time by

the addition of ACh., but thereafter continues. On the other hand, the non-poisoned control *G* shows a considerable increase in respiration on adding ACh. at the 41st min.

It is clear from these two experiments that iodoacetate inhibits practically completely the mechanism which causes an increase of oxidation when ACh. is added, at a time when the resting respiration is still proceeding, though at a diminished rate.

Sodium fluoride. The general layout of the experiments with fluoride was the same as with iodoacetate, except that the fluoride could be placed in the side bulb with the ACh. This is possible because its effect is immediate. The concentration of sodium fluoride in the main vessel after tipping was 0·025 *M*. Table X gives the results of an experiment of this

TABLE X. The effect of fluoride on the respiration with and without the addition of ACh. Figures are Q_{O_2} values of successive 20 min. periods. Those in italics were obtained before emptying the side bulb.

	No addition	With fluoride	With ACh., no fluoride	With ACh. and fluoride
With glucose	12·5	*11·7*	*11·8*	*12·35*
	11·5	12·2	19·7	10·7
	13·0	14·4	19·3	9·8
With lactate	13·3	*14·2*	*17·2*	*16·6*
	13·3	17·7	32·4	15·9
	14·1	17·9	30·0	15·5

kind. The figures show that the resting respiration of the tissue is not affected by fluoride in the concentration used, whereas the increased oxidation normally produced by addition of ACh. is completely suppressed.

Exhaustion experiments

The object of these experiments was to obtain information about the natural substrate of the gland which is utilized in sustaining functional activity. The underlying idea was to exhaust the gland of this substrate, and then determine the effect of adding a known substrate such as glucose or fructose. An attempt was made by administering three 0·5 ml. doses of 0·2 p.c. pilocarpine nitrate to the cat, 5, 3 and 1 hr. before the glands were removed for the manometric experiments. Although this produced profuse salivation, it was not successful in producing any diminution of the ACh. effect as compared with untreated animals. More satisfactory results were obtained by exhaustion of the tissue slices themselves either by allowing them to respire in oxygenated Ringer's solution for several hours or by stimulation with ACh.

In the first of these methods, about twelve tissue slices were placed in a 150 ml. conical flask with 30 ml. Ringer's solution containing 3×10^{-3} *M* bicarbonate without addition of substrate. The flask was filled with oxygen and shaken in the thermostat for 10 hr. After 2, 3 and 5 hr. the Ringer's solution was replaced by fresh fluid. A control slice respired in a manometer during the whole period, being transferred to another manometer with fresh solution after 4 hr. The QO_2 values for this control slice were as follows:

Time (hr.)	1st	2nd	3rd	5th	6th	Next 2 hr. 15 min.	Next 30 min.
Q_{O_2}	10·7	10·7	10·5	9·85	9·8	8·2	7·6

At the end of 10 hr. the transfer of the slices from the Erlenmeyer flask to manometers 2–5 was completed, and the manometric measurements started. The measurements of the control slice (manometer 1) were also continued. The respiration was observed for 20 min., then ACh. was added in manometers 1, 2 and 3, and the observations continued during five successive 10 min. periods. The results of a typical experiment are shown in Table XI.

TABLE XI. The result of exhaustion by long-continued respiration on the effect of ACh. without added substrate and in the presence of glucose and fructose. Figures are Q_{O_2} values.

Manometer	1	2	3	4	5
Contents of side bulb	ACh.	ACh. + glucose	ACh. + fructose	Glucose	Fructose
Initial 20 min.	5·25	8·35	7·5	6·0	6·0
		Side bulb emptied			
Next 10 min.	7·90	19·4	17·2	5·9	5·8
"	Glucose added	18·0	13·7	5·3	5·8
"	9·85	17·4	12·7	5·3	5·6
"	9·00	15·7	11·7	5·2	5·4
"	11·30	15·0	10·2	5·3	5·6

It will be seen from a comparison of the figures for all the manometers for the first 20 min. period and the next 10 min. that, after exhaustion, the exposure of the slices to Ringer's solution containing glucose or fructose causes no increase of the resting respiration. When ACh. is added, the control slice shows a definite but not large increase in oxygen uptake, whereas the slices in contact with glucose or fructose respond in the usual striking fashion, the respiration being more than doubled. The effect of addition of glucose to the control manometer at a later stage of the experiment resulted also in a further increase in oxygen uptake, thus confirming that this sugar was being utilized for the respiration of activity. The slices exposed to fructose showed a more rapid decline in respiration

after addition of ACh. than those in contact with glucose, so that the latter appears to be the more suitable substrate. In the next experiment, exhaustion was attempted by repeated stimulation with ACh.

Preliminary trials showed that the gland tissue did not respond to a second application of ACh. when the first dose was above a certain limit. This finding recalls experiments by Brown & Feldberg [1936], in which it was shown that acetylcholine liberated in a ganglion by preganglionic stimulation can, if protected by eserine, rise to such a concentration that it produces a partial paralysis of the ganglion cells. Also, acetylcholine injected into the arterial tree of a skeletal muscle [Brown *et al.* 1936], or injected into fluid perfusing a ganglion [Feldberg & Vartiainen, 1934], though acting as an immediate stimulus, may be followed by a depression of the responsiveness of the organs.

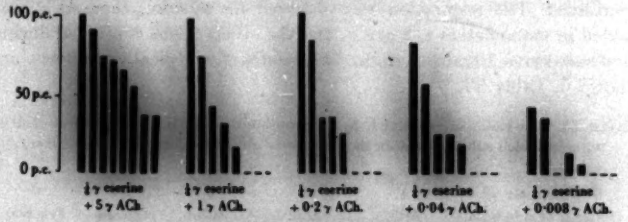

Fig. 2. Comparative effects on the respiration of different amounts of acetylcholine. Each vertical line represents a 10 min. period.

Experiments were carried out, therefore, to find the minimum dose of acetylcholine which would have a maximal stimulating effect. The minimum dose of eserine which has hardly any stimulating effect, but is yet sufficient to protect the acetylcholine, was known from previous work [Deutsch & Raper, 1936] to be 1/12,000,000 (corresponding to 0·25γ in 3 ml.). Slices of tissue were exposed in five manometers to various amounts of acetylcholine, 0·25γ of eserine sulphate being present also. The respiration was observed prior to the addition of the ACh. and in a succession of 10 min. periods afterwards. Fig. 2 shows the percentage increase of the resting respiration produced by addition of ACh. The effect of 5γ ACh. lasts for at least 85 min., whereas with smaller doses it ceases after 55 min. It was decided, therefore, to use a dose of 0·4γ per 3 ml. fluid, protected by 0·25γ eserine for the experiment on repeated stimulation. In this experiment all the slices, immersed in Ringer's solution, were exposed for 1 hr. 45 min. to ACh. simultaneously in an

Erlenmeyer flask filled with oxygen. After the treatment they were transferred to a series of manometers, and the respiration observed for two successive periods of 10 and 15 min. A second stimulation with ACh. was then given and, after noting the respiration during two 10 min. periods, a third stimulation was given to two of the sets of slices (manometers 2 and 3). The observation of the respiration was then continued for a final 10 min. The results are given in Table XII.

TABLE XII. Effect of a single stimulation with ACh. on the response to subsequent addition of ACh. without added substrate and in the presence of glucose (0·2 p.c.) and fructose (0·2 p.c.). The figures are Q_{O_2} values.

Manometer	1	2	3	4
Substrate	Glucose	Fructose	Fructose	None
Initial 15 min.	9·7	8·1	8·2	6·0
Next 10 min.	10·1	8·4	7·6	4·8
	Stimulation with 0·4γ ACh. in all manometers			
"	27·4	9·4	9·7	4·8
"	27·0	8·4	9·2	4·8
		0·4γ ACh. added	0·8γ ACh. added	
"	23·4	10·5	10·2	2·7

It will be observed that, after the first exhaustion period, the respiration is greater when the slices are placed in contact with glucose or fructose than in the absence of added substrate. The second addition of ACh. produced a very large increase of oxygen consumption in the presence of glucose, but hardly any effect in the presence of fructose. With no added substrate the ACh. had no effect. The slices in contact with fructose were given a third stimulation with ACh., but it was no more effective than the second stimulation.

The results of these two exhaustion experiments show that the resting respiration in a period of 10 hr. exhausts the gland partially of the substrate available for producing the increased oxygen uptake provoked by ACh. A just maximal stimulation with ACh. exhausts the gland substance of this substrate completely. In the tissue exhausted by the latter method, the resting respiration is greater when glucose or fructose is added, and of the two glucose has the greater effect. Again, when stimulated by ACh., the fully exhausted tissue will give a maximal response if glucose is present and a much smaller one with fructose. With no added substrate, ACh. has no effect. It can be concluded, therefore, that the gland can be stimulated when either glucose or fructose is present, but fructose is much less effective for maintaining activity. It

cannot, however, be concluded that the gland can only be stimulated in the presence of these particular substrates, because others may be removed by the procedure of exhaustion.

The respiratory quotient

The R.Q. was determined in Dixon-Keilin manometers. With this method it is not possible to measure the R.Q. of a set of tissue slices, and then of the same set after adding ACh. Comparative experiments only can be carried out. The metabolism of the tissue at rest was observed usually over 1½–2 hr., whereas that of activity was limited to a period of 45 min. This was necessary in order to avoid an overlapping of the two, and it has been shown above that the stimulating effect of ACh. in maximal dosage lasts for longer than 45 min.

The experiments yielded the following results. When no substrate was added the R.Q. varied between 0·59 and 0·8 when the tissue was either in the resting state or stimulated by ACh. The same figures were obtained when fructose was added, except in one instance when the R.Q. was unity. On the other hand, the addition of glucose caused the R.Q. during activity to rise to 1·02. These results show that in activity the gland can use other substrates as well as carbohydrate, though the latter is used preferentially when present.

The inhibitory effect of atropine

The current opinion regarding the mode of action of acetylcholine *in vivo* is that acetylcholine is liberated by nerve impulses, each portion acting as a stimulus until it is destroyed by acetylcholine esterase. If

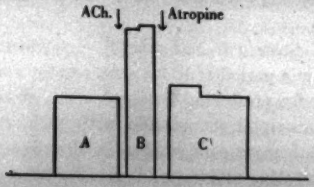

Fig. 3. Effect on respiration of atropine added after acetylcholine. A, resting tissue. B, after addition of ACh. C, after subsequent addition of atropine.

this be true of the action of ACh. on slices of salivary gland *in vitro*, then atropine *following* the addition of ACh. should annul its action. As shown in Fig. 3 this is what occurs. The dependence of activity on the continued presence of acetylcholine suggests that, when added to the tissue, it does

not cause an extensive liberation of oxidizable substrate which can then be oxidized by the oxidation mechanisms present in the gland independently. There thus appears to be a quantitative relationship between the amount of acetylcholine added and the increased oxygen uptake which it causes.

Discussion

In their studies of the utilization of blood sugar by the dog's submaxillary gland, Anrep & Cannan [1922, 1923] showed that sugar was removed from the blood by the resting gland, and that the rate of removal was not affected by atropine. Administration of pilocarpine or chorda stimulation caused an increase in this removal of sugar and the increase was not due to passage of sugar into the lymph. This indicated an increased sugar consumption by the gland itself during activity [Anrep, 1923], because the sugar does not appear in the mucin secreted nor as free glucose in the saliva. The excess sugar consumption of activity disappears as soon as chorda stimulation ceases [Anrep & Cannan, 1923]. These results suggest that sugar is taken up by the gland from the blood to maintain the secretory activity, and they render it probable that there is not a large reserve of substrate in the gland to provide the necessary energy for the secretory process.

Our own results, obtained by different methods, have also shown the ability of the gland tissue to make use of glucose in activity. They have, however, allowed a more detailed analysis of the metabolism both of rest and of activity to be made. Although the experiments have been carried out with the isolated tissue, and respiratory phenomena and glycolysis only have been studied, we have no reason to doubt that they may be used to interpret the behaviour of the gland *in vivo*.

It is clear that the gland possesses its own substrates which can serve for the oxidation occurring both in rest and activity. Some of this substrate may be carbohydrate, but other substances are utilized too, as the R.Q. experiments testify. It has been shown that this substrate may be removed by exhaustion, and then stimulation by ACh. does not increase the respiration. If, however, a suitable substrate, such as glucose, is now made available, ACh. gives its maximal stimulating effect on the respiration. It seems likely, therefore, that one reason for the large increase in blood flow which occurs in the active gland *in situ* is to provide glucose for the maintenance of activity. At the beginning of a period of activity the substrate in the gland will serve for the supply of energy, but probably not for long. We have not been able to find any evidence that anaerobic processes can supply energy for the secretion

process because ACh. has no effect on the tissue under anærobic conditions. This is in marked contrast to the behaviour of skeletal muscle, which can obtain energy for contraction for considerable periods of time under anaerobic conditions.

The aerobic glycolysis which occurs for a short time after stimulation of the tissue with acetylcholine is probably not of importance under the natural conditions of stimulation in the body. We regard it as due to the special conditions existing in experiments by the tissue slice method where the supply of oxygen cannot be as efficient as in the gland with its circulation intact.

The effect of iodoacetate and fluoride, which inhibit the effect of acetylcholine although the resting respiration is still active, shows that the resting respiration is qualitatively different from the respiration of activity, otherwise both would be inhibited by these two agents. The tissue is still able to burn lactate in the presence of either of these substances. In general there are two ways in which their inhibitory effect may be produced. The first is interference with the stimulating agent itself or its site of action, that is, an atropine-like action, since this is the current view of the way in which atropine exerts its effect. The second is interference at some later stage with the series of reactions induced by the application of the stimulating agent. This is the commonly accepted view of the mode of action of iodoacetate and fluoride. On the available evidence we cannot exclude the first of these alternatives, but it does not appear to us as a probable explanation. Regarding the second view, it may be pointed out that although the experiments show that these two agents may be acting by inhibiting glycolysis, they must in addition be interfering with the processes responsible for that extra oxygen uptake of activity which is observed when the stimulated tissue is using its own reserve substrate. One must admit, therefore, that other mechanisms besides glycolysis are being inhibited by the fluoride and iodoacetate, although their nature is not yet known.

An indication of the nature of the response of the submaxillary gland to stimulation by ACh. is given by the experiment in which atropine was added whilst the increased oxygen uptake caused by ACh. was still marked. Atropine abolished this excess oxygen consumption. If the increased oxidation provoked by ACh. were due to a sudden liberation of oxidizable substrate which could then be oxidized by the oxidation mechanisms of the gland cells, it should not be inhibited by atropine added after the ACh. It is evident that the increased oxygen consumption is, therefore, more likely to be concerned with the active secretory

process than with an oxidative recovery phase such as has been demonstrated in frog's skeletal muscle following contraction. This supplies confirmatory evidence to that already given that the energy immediately required for the secretory process is dependent on aerobic and not anaerobic chemical processes. It suggests that the control quantitatively of respiration accompanying secretory activity is determined by the acetylcholine liberated on stimulation of the secretory nerve.

On the whole the facts show a much closer resemblance of the metabolism of submaxillary gland tissue to that of nerve fibre than to that of voluntary muscle.

Summary

1. The respiration of thin slices of the surviving submaxillary gland of the cat has been investigated under a variety of conditions.

2. Observation of the resting tissue revealed the following:

(*a*) The R.Q. in Ringer's solution varies between 0·59 and 0·8.

(*b*) The substrate in the gland which provides for the resting respiration may be partially exhausted by allowing the tissue to remain in oxygenated Ringer's solution for several hours or by the action of a single suitable dose of acetylcholine.

(*c*) In tissue exhausted by the latter method the respiration is increased slightly if glucose or fructose be added.

(*d*) Iodoacetate causes progressive diminution of the resting respiration with time in presence of glucose, but increases it at first in presence of lactate.

(*e*) Fluoride has no effect on the resting respiration in presence of glucose but increases it in presence of lactate.

(*f*) Under anaerobic conditions and with no added substrate there is a weak glycolysis. Glucose increases this but not fructose.

3. When acetylcholine, with a minimum amount of eserine, is added to the resting tissue, a considerable increase in respiration occurs. This respiration of "activity" displays the following characteristics:

(*a*) The R.Q. without added substrate is similar to that of resting tissue but is raised to 1·0 in the presence of glucose.

(*b*) In the first few minutes following addition of acetylcholine, an aerobic glycolysis occurs if glucose is present. Chemical estimation of the lactic acid produced accounts for about half that expected from manometric determination. No aerobic glycolysis under the same conditions is observed if the glucose is replaced by pyruvate or fructose or with no added substrate.

(*c*) Ethylcarbylamine prolongs the period of aerobic glycolysis.

(*d*) Exhausted tissue [see 2 (*b*)] subjected to the action of acetylcholine displays only a small or no increase in respiration, but a maximal increase is obtained if glucose be present. Fructose under the same conditions produces only a slight effect.

(*e*) Iodoacetate or fluoride abolishes completely the respiratory effect of acetylcholine when the resting respiration is still active.

4. The anaerobic glycolysis of the tissue is unaffected by acetylcholine.

5. Atropine added either before or after acetylcholine inhibits completely the effect of the latter.

6. Adrenaline acts like acetylcholine in causing a preliminary aerobic glycolysis in presence of glucose.

7. Atropine has no effect on the respiratory increase produced by adrenaline.

8. It is concluded (*a*) that the characteristics of the resting respiration of the gland are qualitatively and quantitatively different from those of the stimulated tissue, and (*b*) that the liberation of energy for purposes of secretion is brought about by oxidative processes depending on the continual presence of acetylcholine and that the reserves of the tissue which are oxidized are not solely carbohydrate. On the other hand, glucose and, to a small extent, fructose, if added, are preferentially utilized.

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THE EFFECT OF LARGE DOSES OF INSULIN ON THE FŒTAL SHEEP AND GOAT

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Unlike most animals sheep and goats have a higher glucose level in the fœtal blood than in the maternal. It was of interest, therefore, to investigate how far the facts concerning the fœtal carbohydrate metabolism in dogs, which were discussed in a previous paper [Schlossmann, 1938], hold true for the fœtal lamb and goat.

Methods

Blood sugar was determined by Hagedorn & Jensen's method, and lactic acid by that of Friedemann, Cotonio and Shaffer.

The abdomen and uterus of the ewes and goats were opened and the fœtuses delivered into the saline bath at 37° as described previously [Schlossmann, 1932, 1938].

Results

Initial blood-sugar levels of mother and fœtus

Table I shows the initial blood-sugar levels of mother and fœtus. The fœtal are distinctly higher than the maternal levels in all cases except the last, where that of the mother was abnormally high.

It can be seen from the data collected by Needham [1931, Table 238] that in man, dog, rabbit, guinea-pig and rat the levels of the blood sugar are higher in the maternal than in the fœtal blood; whereas a higher level in the fœtal blood has only been found by Aron [1924] in the cow and pig. As a general rule then, the blood-sugar level is higher in the maternal blood, except in ungulates.

In previous papers [Schlossmann, 1930, 1932] the regulation of the passage of glucose from the mother to the fœtus was considered to be due to the difference in the sugar levels of the maternal and fœtal blood,

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and a secretory activity of the placenta had been denied. The finding of the higher blood-sugar level in the fœtus of ungulates necessitates some other factor besides the sugar level in the maternal blood which determines the passage to the fœtus in these animals. We cannot at present give a satisfactory explanation of this mechanism which is peculiar to ungulates.

The differences in the blood-sugar levels of the umbilical artery and vein (Table I) are usually small. In Exp. 5 maternal blood was taken by puncturing the small arteries and veins which go to and come from the

Table I. Glucose content of the maternal and the fœtal blood in pregnant sheep and goats at the beginning of the experiments. All animals were near term. Figures in mg. per 100 ml. Intravenous anæsthesia with urethane (Exps. 1–5) or pernocton (Exps. 6 and 7).

		Mother		Fœtal blood sugar Blood taken from		
No.		Blood from	Blood sugar	Umbilical artery	Umbilical vein	Carotid artery
1	Sheep	Jugular vein	62	71	76	—
2	„	„ „	88	90	98	—
3	„	Carotid artery	112	—	—	188
4	„	„ „	110	—	—	172
5	Goat	Uterine artery	105	160	156	—
		Uterine vein	85	—	—	—
6	„	Jugular vein	105	184	184	—
7	„	„ „	183	183	180	—

cotyledons. Here the difference in the blood-sugar levels between arterial and venous blood was 20 mg. p.c., and this difference remained constant over 3 hr. This shows that a comparatively large amount of sugar is taken up by the cotyledons. But the mechanism of the glucose passage through the placental barrier is made no clearer, as the amount and the glucose content of the blood inside the cotyledons is unknown. A part of this blood might be stagnant and more or less out of the normal circulation.

Effect of glucose infusion

The placenta in the dog is permeable to glucose [Schlossmann, 1932]. Considering the higher glucose level of the fœtal blood in sheep it was necessary to determine whether the same is true in these animals. 200 ml. of a 20 p.c. glucose solution were infused into the jugular vein of an ewe near term, the infusion lasting 3 min. The rapid rise in the maternal blood sugar was followed by a less sharp rise in the fœtal blood sugar (Fig. 1). After 25 min. the two curves crossed over, and there was a gradual fall to the normal level, the fœtal values being above the maternal. The result is exactly the same as that obtained previously in

dogs, and it is only consistent with the view that—at least under these experimental conditions—the placenta is permeable to glucose in both directions (Hoeber's "physikalische Permeabilität").

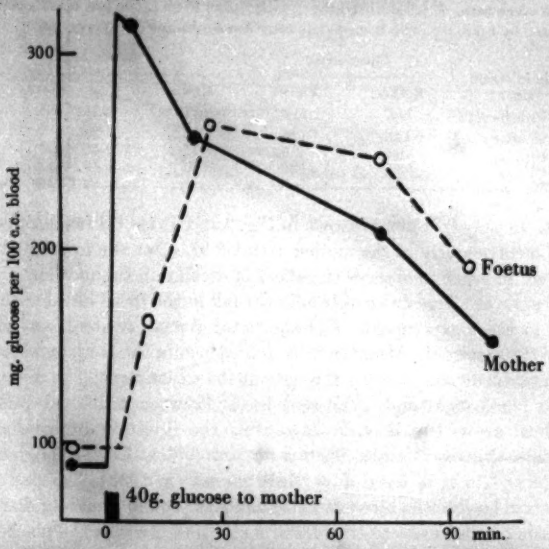

Fig. 1. Experiment 17. iii. 36. Sheep tupped 26. x. 35. Weight of the ewe 38·2 kg., of the fœtus 3·85 kg. Urethane anæsthesia intravenously. 200 ml. of 20 p.c. glucose infused to mother intravenously within 3 min. Maternal blood was taken from jugular vein, fœtal blood from umbilical vein.

Effect of insulin on fœtus and mother

After opening the uterus and delivering the fœtus into the saline bath, samples of blood were taken from the mother and the fœtus. Insulin was then given to the fœtus intravenously, and other samples of blood were taken after suitable intervals. In the first experiment 200 units of insulin were injected intraperitoneally into the fœtus without effect on the fœtal and maternal blood-sugar levels. The result of another experiment is shown in Table II. After the injection of 300 units of insulin the fœtal blood-sugar level dropped from 188 to 126 mg. p.c., and the lactic-acid level increased slowly. Shortly before death a small increase of the blood-sugar and a sudden rise of the lactic-acid level was

TABLE II. Experiment 3. iii. 37. Weight of the ewe 34·1 kg. Urethane intravenously. Fœtus 131 days old, weight 2·5 kg. Blood samples from maternal and fœtal carotid artery. 300 units of insulin (Hoechst) injected intravenously into the fœtus. Four hours after the injection the umbilical cord was tied and the lamb was removed from the saline bath. It failed to breathe and died after 15 min. The last blood sample was taken by heart puncture immediately after death. Values in mg. per 100 ml.

Insulin injection min.	Blood sugar		Lactic acid	
	Mother	Fœtus	Mother	Fœtus
10 before	112	188	11	15
90 after	117	126	15	29
180 „	85	138	11	45
240 „	84	128	10	49
255 „	—	142	—	99 Fœtus dead

found. In the experiments shown in Fig. 2 and Table III insulin was also given intravenously to the mother about 2 hr. after the fœtus had been injected, in order to observe the effect of insulin on the mother.

Despite the large doses of insulin the fall in the fœtal blood sugar was small in these experiments, and the fœtal glucose content was always above the maternal. After the injection of insulin the lactic-acid level of the fœtal blood rose steadily throughout the experiment. The differences of the blood-sugar and lactic-acid levels between umbilical vein and umbilical artery—marked in dogs after the injection of insulin—are insignificant in fœtal lambs. There is no such evident fœtal hypoglycæmia to compensate as in fœtal dogs [Schlossmann, 1938]. The rise of the lactic-acid level in the blood of fœtal lambs is probably mainly due to an increasing impediment to transfer of lactic acid through the placenta to the mother. Fœtal lambs and goats are much more resistant to insulin than fœtal dogs, the insulin doses being nearly the same in these experiments (60–415 units per kg.) as in those on dogs (65–235 units per kg.).

After tying the umbilical cord and removing the fœtus from the saline bath a sudden rise in the blood-sugar and lactic-acid levels was observed (Table II and Fig. 2). The rise in the lactic-acid level may be explained by the fact that after birth no more lactic acid can pass across the placental barrier to the mother. The rise in the blood sugar is more difficult to understand, considering the large amounts of insulin injected into the fœtus. Perhaps the mobilization of glycogen from the fœtal stores is due to the cooling after removal from the saline bath.

The lamb into which 1200 units of insulin had been injected was still alive 6 hr. after birth, and in no experiment were convulsions observed. The adult sheep, too, is comparatively resistant to insulin. Strand *et al.* [1934] injected 2–3 units of insulin per kg. of body weight into four

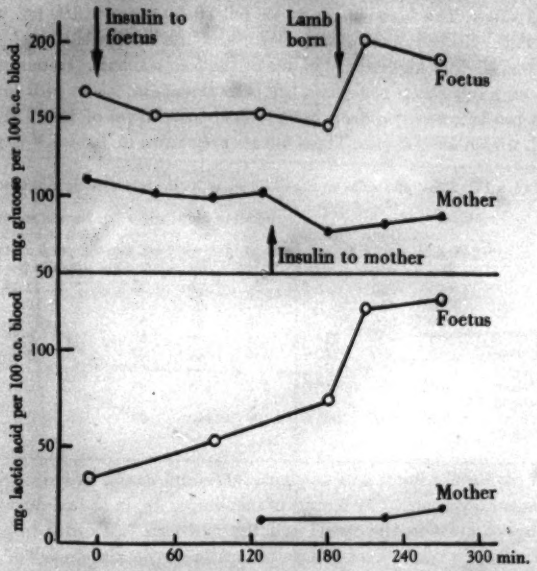

Fig. 2. Experiment 11. iii. 37. Weight of ewe 30·0 kg. Spinal anæsthesia with durocain, 2 hr. later 5 ml. dial intravenously. Weight of fœtus 3·18 kg., 139 days old. 1200 units of insulin (Hoechst) were injected intravenously into the fœtus, 135 min. later 150 units intravenously into the mother. 185 min. after the insulin injection into the fœtus the umbilical cord was tied and the lamb removed from the saline bath. Blood samples were taken from maternal and fœtal carotid artery. The maternal figures are represented by dots, the fœtal by circles.

TABLE III. Experiment 19. iii. 37. Weight of the goat 45·0 kg. Urethane intravenously. Fœtus 142 days old, weight 2·95 kg. Blood samples from uterine artery and umbilical artery. 600 units of insulin (Hoechst) were injected intravenously into the fœtus, and 2 hr. later 250 units intravenously into the mother.

Insulin injection into fœtus, min.	Blood sugar mg. per 100 ml. Mother	Fœtus	
10 before	105	160	
55 after	103	140	
120 „	99	140	Insulin to mother
180 „	101	163	
240 „	80		

normal sheep. The blood-sugar levels fell 20–30 mg. per 100 ml. within 45–90 min. No convulsions were seen, and the lactic-acid level of the blood was slightly higher only in one of the four animals. In our experiments on adult sheep and goats the intravenous injection of 5 units of insulin per kg. caused a drop in the blood-sugar level of 18–37 mg. per 100 ml. within 45–100 min. These figures are shown in Table IV together

Table IV. Effect of insulin on the blood-sugar level. Intravenous injection

	Weight kg.	Units injected	Units per kg.	Fall of blood sugar			Insulin sensitivity (fall of blood sugar per 5 units of insulin per kg.)
				From	To	mg. per 100 ml.	
Goat	45·0	250	5·5	99	80	19	17·0
Ewe	30·0	150	5·0	101	83	18	18·0
Ram	57·0	285	5·0	78	41	37	37·0
Lamb (24 hr.)	2·6	300	115·0	111	49	62	2·7
Fœtal sheep (131 days)	2·5	300	120·0	188	126	62	2·6
Fœtal sheep (139 days)	3·2	1200	415·0	172	148	24	0·3
Fœtal goat (142 days)	2·95	600	202·0	160	140	20	0·5

with those for the fœtal and newborn lamb and goats. The drop in the blood-sugar level caused by 5 units of insulin per kg. in the adult requires hundreds of units in the fœtus and the newborn. In order to get an indication of the relative sensitivity to insulin the maximum fall of blood sugar has been divided by each 5 units of insulin given per kg. of body weight. The figures (last column of Table IV) give no more than a rough idea, as they are based only upon the few experiments described above, and upon the observation that doses of 5 units per kg. have no effect on the blood sugar of the fœtal lamb.

Effect of fœtal lamb plasma on the action of insulin in the rabbit

It has long been known that certain cases of diabetes are peculiarly resistant to insulin. Recently de Wesselow & Griffiths [1936] have shown that the plasma from these diabetics, when injected into young rabbits, has an inhibitory effect on the normal action of insulin. They suggest that these patients have in their blood a substance with a diabetogenic action, possibly of pituitary origin, which antagonizes the action of insulin. We considered that the remarkable resistance of the fœtal lamb to insulin might be due to a similar substance.

To test this the response to an intravenous injection of 0·2 unit of insulin per kg. into rabbits weighing between 1 and 2 kg. was determined,

TABLE V. Rabbits fasting for 18 hr. Maximum observed fall in blood sugar as percentage of the initial blood-sugar level after intravenous injection of 0·2 unit of insulin per kg.

Rabbit	Insulin alone	Three hr. before 10 ml. horse serum	Three hr. before 10 ml. fœtal lamb plasma
1	58	—	32 (4 ml. only)
2	55	36	15
	52		
3	30	31	11
4	39	25	21

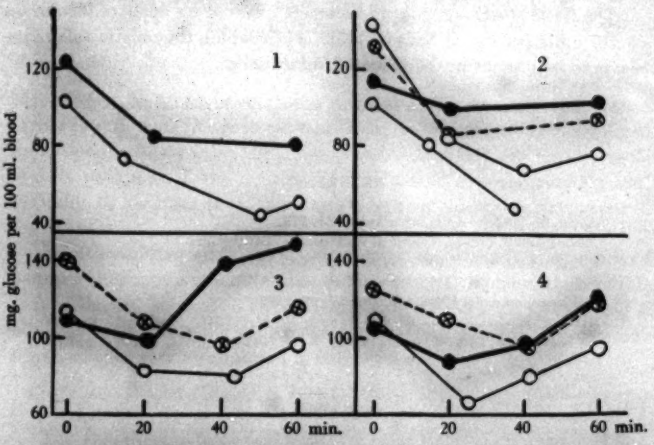

Fig. 3. Four rabbits, blood sugar in mg. p.c. ○——○ = 0·2 unit of insulin per kg. injected intravenously. ●——● = 10 ml. of fœtal lamb plasma (only 4 ml. in Exp. 1) injected subcutaneously, 3 hr. later, insulin as above. ⊗- - -⊗ = 10 ml. of normal horse serum injected subcutaneously, 3 hr. later, insulin as above.

according to the method of de Wesselow & Griffiths. A few days later the rabbits received subcutaneously 10 ml. of fœtal lamb plasma and 3 hr. later the standard dose of insulin intravenously. The insulin test was repeated a third time 3 hr. after 10 ml. of normal horse serum had been given subcutaneously as a control. Fig. 3 shows the results, and in Table V the maximum falls in the blood-sugar levels of all tests are collected. It will be seen that the fœtal lamb plasma always had a marked inhibitory effect, and in two of the four cases a small drop in the blood-sugar level was followed by an increase above the initial level. In two cases, however, horse serum had a similar though smaller effect.

Although these experiments are few, and not too well controlled, they indicate that the presence in the blood of a substance actively antagonistic to insulin might play a part in the resistance of the fœtal lamb to insulin.

Summary

In the sheep and the goat the blood-sugar levels are substantially higher in the fœtus than in the mother. The permeability of the placenta to glucose is shown by infusing glucose into the mother.

The fœtal sheep and goat are scarcely affected by doses of insulin up to 415 units per kg. of body weight. An insulin-antagonistic substance seems to be present in the blood of fœtal lambs.

We wish to thank Sir Joseph Barcroft for his interest and his help in this work.

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COUGHING STUDIED BY MEANS OF X-RAY CINEMATOGRAPHY

By K. J. FRANKLIN and R. JANKER

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(*Received* 1 *March* 1938)

The studies to be described below were made in connexion with some earlier experiments [Barclay *et al.* 1938] upon the excretion of insufflated radio-opaque dusts from the lungs of cats. Our primary objects were, first, to obtain an X-ray cinematographic record of such excretion and, secondly, to see if we could find any evidence of peristaltic movement in the bronchi.

As a fortuitous addition to our results, we secured a sequence of pictures illustrating acts of coughing. The data yielded by analysis of this part of the film seemed to us to be worthy of a brief note, as coughing is not only a common and important pathological response, but also one about which extra information is still needed.

Methods

The experiments were performed by us at Bonn upon a series of four cats, but most of the results to be mentioned were obtained from the second one, so we shall content ourselves here with a description of the procedures adopted in the case of that animal. It was first given an intramuscular injection of 1 in 10,000 atropine sulphate solution in the dosage of 0·2 c.c. per kg. Then, after an appropriate interval, it was deeply anæsthetized with a mixture of 1 part of chloroform to 2 parts of ether. Next, a suitable quantity of very finely powdered lead glass was insufflated into the lower lobe of the left lung by means of a technique that has already been described in detail elsewhere [Barclay *et al.* 1938]. An hour after the injection of the atropine sulphate solution, the first record was taken (30 sec. at 17 frames per sec.) with the subject in the left lateral position; the X-ray cinematographic apparatus used was Janker's animal one [Janker, 1932, 1933], and the method was the

indirect one. Following this, the cat was given an intraperitoneal injection of 5 p.c. nembutal solution in distilled water in the dosage of 0·5 c.c. per kg. Three further X-ray cinematographic records were taken at hourly intervals, and between the taking of the second and third of these the animal was given another injection of nembutal solution by the same route and in the same dosage. The fifth, and last, X-ray cinematographic record was taken two hours after the fourth one, and thereafter the animal was killed.

The 35 mm. proof-film was subjected to examination at Oxford. It was first projected a large number of times, until all the salient points had been noted. Then the third section of the record of the second cat, which contained the best pictures of coughing, was excised from the main film and examined in greater detail. During the period under consideration, the cat had in succession respired normally once or twice, coughed twice, respired normally for a longer period, coughed three more times, respired normally for a short period, and finally coughed once again. The first, second, and fourth acts of coughing were selected for analysis by means of outline drawings made from enlarged stills. The projector (Zeiss Ikon Kinobox CK 600) was so arranged that individual frames gave vertical pictures about 25 cm. wide on sheets of paper. The frames showing maximum inspiration and expiration in each of the three acts of coughing were then projected in succession, and outline drawings made of the essential features. These frames may for convenience be called I *i*, I *e*, II *i*, II *e*, III *i*, and III *e*. The intervals between I *i* and I *e*, I *e* and II *i*, II *i* and II *e*, and III *i* and III *e*, were respectively 12, 22, 12, and 7 frames, i.e. 0·7, 1·3, 0·7, and 0·4 sec. Finally, a combined outline drawing of II *i* and II *e* was made on a single sheet of Bristol board for the purpose of a comparative study, and separate outline drawings of II *i* and II *e* were made on a second sheet of Bristol board for purposes of reproduction (Fig. 1). The drawings for analysis were made with very finely pointed pencils, and the drawer's opinion of the outline of each shadow was agreed by an independent observer before it was accepted. The drawings reproduced in illustration of this paper were first done in pencil in the same way, and then inked in with Indian ink; the process inevitably caused a loss of some detail. In all the pencil work the outlines of the main bronchus, and of the entries and bifurcations of its tributaries, were drawn with maximal accuracy; the outlines of the tributaries themselves, apart from the special points mentioned, were delineated with less care, as they were not of importance for the calculations that were planned.

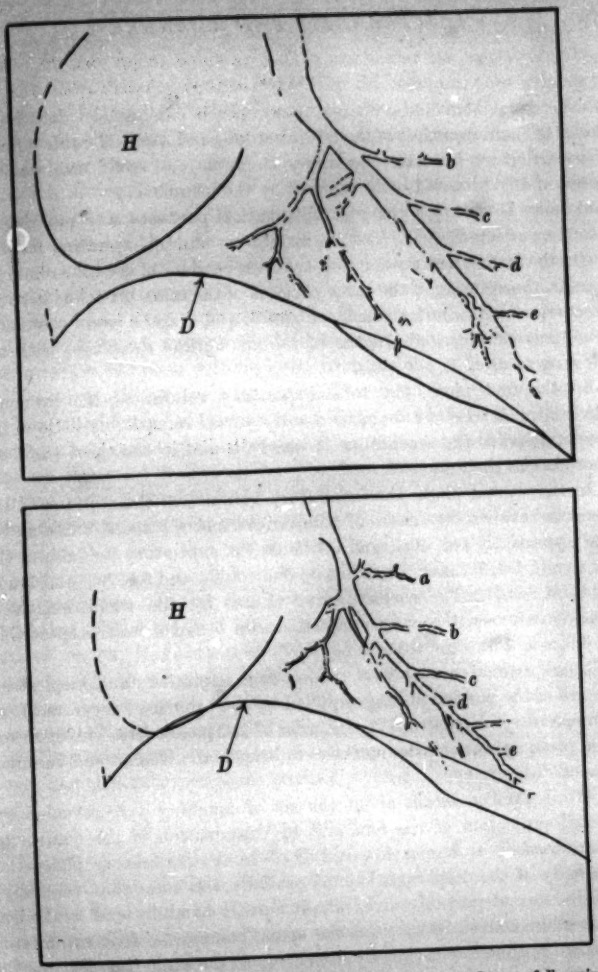

Fig. 1. Upper: full inspiration during an act of coughing in a cat. Lower: full expiration, 0·7 sec. later. The cat was lying in the left lateral position. The figures show the outlines of the main bronchus, and of certain of its tributaries, in the lower lobe of the left lung. *H*, outline of heart shadow. *D*, outline of rostral diaphragm shadow. The rectangles enclose corresponding areas from the two frames of the film.

Results

The insufflated radio-opaque powder was adequately distributed along the main bronchus of the left lower lobe and a considerable number of its tributary bronchi. Nowhere, so far as one could see, was the lumen of any bronchial tube blocked by the insufflation.

It may be assumed that, for all practical purposes, any radiological distortion affected equally both the inspiratory and the expiratory records.

On the further assumption that the cross-sections of the tube remained circular, the volumes of the three portions of the main bronchus between the entries of tributaries *b* and *c*, *c* and *d*, and *d* and *e*, were calculated from their calibres and lengths in the six outline drawings, with the following results.

In the first place, the total expiratory volume of the bronchus between the entries of tributaries *b* and *e* varied remarkably little in the three coughs; in the second one it was 1·01, and in the third one 1·04, times that in the first one.

In the second place, the inspiratory volumes of the sections of the bronchus between the entries of tributaries *b* and *c*, *c* and *d*, and *d* and *e*, were respectively 6·3, 10·1, and 6·7 times the expiratory volumes in the first cough, 5·9, 7·1, and 7·3 in the second cough, and 5·8, 8·2, and 9·5 in the third cough. The corresponding figures for the whole length of bronchus between the entries of tributaries *b* and *e* were respectively 7·4, 6·6, and 7·3 in the three coughs.

Rough estimates, that were made of the expiratory and inspiratory volumes of the portion of lung supplied by the bronchus, suggested that the inspiratory increase was of the order of 200 p.c. or less. This increase would presumably comprise increases in bronchial, alveolar, and vascular volumes.

Certain further details about the act of coughing were revealed by repeated projection of the film and by examination of the composite outline drawing of frames II *i* and II *e*. In the inspiratory phase the main body of the diaphragm moved caudally and somewhat ventrally, while its sternal portion moved about equally caudally and ventrally. The sternum moved away from the spinal column, so that the lateral width of the thorax was thereby increased; at the same time the middle portions of the ribs moved outwards and rostrally, so that their shadows in the lateral view became straighter. The heart and the bifurcation of the trachea moved with the sternum away from the spinal column, and were also drawn caudally a short distance. The greater part of the thoracic

portion of the spinal column, and in particular its upper vertebræ, moved a short distance farther away from the sternum, thus helping to increase the lateral width of the thorax; the two or three lowest thoracic vertebræ, on the other hand, moved in the opposite direction. The whole gave the impression of a process akin to the tensing of a strong bow, and the quick spring back with the onset of expiration heightened this impression. As a result of the various inspiratory movements mentioned above, the axis of the main bronchus of the left lower lobe described a line that was convex towards the spine, in place of an expiratory line that was concave towards it. Directly inspiration gave way to expiration, there was a very rapid reversal and, although full expiration took 12, 12, and 7 frames respectively in the three coughs, the process was in large measure complete within $\frac{1}{17}$ sec. from the end of inspiration.

Two other points may be mentioned in connexion with coughing; the first was noted during projection of another section of the Bonn film, the second during radiological screening in an earlier, unpublished experiment by Barclay & Franklin. The former illustrated the effect of coughing upon a foreign body, which is lying upon the surface of a bronchus but does not seriously impede the movement of air within it. Some lead glass dust, that was in such a position, was carried upwards for some distance by a cough, and did not move back during the subsequent inspiration. It was thus removed from its original site near the bifurcation of the trachea, which is the second most sensitive "tussiceptive" region [Barclay *et al.* 1938], to a less sensitive one higher up, and the coughing stopped. In Barclay & Franklin's experiment, on the other hand, a capsule of bismuth, placed at the far end of the trachea of an anæsthetized cat, occupied so great a proportion of the lumen that it was carried nearly to the larynx during the expiratory phase of a cough, and then returned to its starting point in the subsequent inspiration.

Discussion

The only point which we wish to emphasize is that, if we were correct in our assumption that the cross-sections of the bronchi remain circular, then in the inspiratory phases of the acts of coughing there was a sixfold increase in volume, due mainly to an increase in cross-section, of the main bronchus studied. As the volume of the portion of the lung that included this bronchus increased at most, according to our estimate, by 200 p.c., and this increase probably included one in blood capacity, it would appear that the inspired air was accommodated in the expanding bronchi to a far greater extent than in the expanding alveoli.

SUMMARY

1. The volume changes of the main bronchus of the left lower lobe of a cat's lung were calculated from X-ray cinematographic records of a series of coughs.

2. The expiratory volume of this bronchus was remarkably constant throughout the series.

3. The inspiratory volume was to the expiratory as 7 to 1.

4. The inspiratory volume of the portion of lung containing the bronchus, on the other hand, was to the expiratory as not more than 3 to 1, according to our estimate.

5. Certain further details are given about the act of coughing, as recorded by X-ray cinematography and observed during screening.

The English joint author of this paper wishes to express his thanks to Prof. Frhr. v. Redwitz, Director of the University Surgical Clinic, Bonn, and to Prof. Robert Janker and his assistants in the Radiological Section of the Clinic. He is indebted to Mr Maurice Tuckey, technician to the Nuffield Institute, for help during the analysis of the film. The copyright of the film is held by Reichstelle für den Unterrichtsfilm, Berlin; we are indebted to it for permission to export the proof-film for analysis, and to H.M. Customs and Excise for permission to import it duty free.

Since the film was made, Prof. Robert Janker has left the University Surgical Clinic for Röntgen- und Licht-Institut, Bonn, Baumschulallee 14.

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The relation of the vagus nerve to the ductus arteriosus in the guinea-pig. By JOSEPH BARCROFT, J. A. KENNEDY and M. F. MASON

The guinea-pig offers certain advantages for the above study:

(1) Unlike the rabbit, its fœtus is born in a mature condition.

(2) Owing to the smallness of the vessels and consequent transparency of their walls, a judgment can be made of the condition of the lumen from the colour.

This is important because the muscle of the ductus, being disposed in diagonal bands [v. Hayek, 1935, confirmed by Boyd, 1936], can close the lumen without producing much change in the external diameter.

1. (*a*) Stimulation of the peripheral end of the left vagus produces blanching of the ductus, especially that portion nearest the aorta—also slowing of the heart.

(*b*) When the heart "escapes" or the stimulation ceases, with consequent cardiac acceleration, the ductus does not immediately appear to relax though the pressure in the pulmonary trunk returns.

2. Stimulation of the peripheral end of the right vagus produces an uncertain effect; if there is any constriction of the ductus it is nearer the heart than that produced by the left vagus.

3. The slowing of the heart usually takes place with both vagi.

4. In one fœtus, in which there was little or no slowing of the heart on stimulation of either vagus, the right vagus did not close the ductus, whereas the left vagus did.

5. Stimulation of the central end of the right vagus does not close the ductus with the left vagus intact.

6. On perfusion of the ductus with Ringer's solution, in three experiments addition of pilocarpine produced some degree of constriction. In the experiment in which the pilocarpine had the strongest effect, atropine failed to open the ductus. In the other two atropine opened the ductus.

Two *caveats:*

(1) The above statement does not rule out the possibility of an additional local mechanism.

(2) Pending further experiments the statement applies only to guinea-pigs.

We would like to thank the National Research Institute for providing us with stock at an inconvenient season of the year.

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The isolated heart circuit. By L. N. KATZ and M. MENDLOWITZ

With the circuit described in Fig. 1 we were able to measure the right and left arterial blood pressures and resistances, the right and left

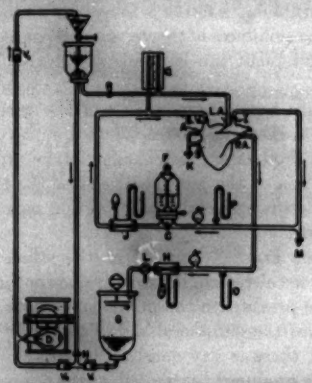

Fig. 1. Diagram of the circuit. *P.A.* cannulated left pulmonary artery. *I*, cannulated innominate artery. *L.A.* cannula into left auricle. *S.V.* cannulated superior vena cava. *A*, cannulated azygos vein. *S*, cannula in coronary sinus via inferior vena cava. *M* and *K*, sampling tubes. *P* and *O*, mercury manometers. *J* and *H*, resistances. *L*, three-way stopcock for measuring pulmonary flow. *F*, modified Ludwig stromuhr for measuring aortic flow. Pinch-clamp *C* is removed except when aortic flow is being measured. *Q*, water manometers. *G*, aerator enclosed in a thermostatic cabinet. *B*, reservoir enclosed in a thermostatic cabinet. The inflow from the funnel, at the bottom of which are glass beads acting as a foam-trap, is regulated by means of a screw-clamp in such a way as to keep the level of the blood in the reservoir constant. A thermometer records the temperature of the reservoir blood. *D*, pump keeping the reservoir blood circulating and mixed. *N*, pinch-clamp applied as necessary in order to drain blood from *G*. *R*, screw-clamp regulating inflow. ↓, direction of blood flow. V_1, V_2, V_3, valves.

auricular pressures, the right and left heart outputs, heart rate, diastolic volume (oncometer), blood temperature, arterio-venous oxygen difference

of the heart, and alterations in the level of blood sugar, lactic acid and plasma carbon dioxide content during the course of the experiment. We also determined indirectly the total coronary flow (pulmonary minus aortic flow), oxygen consumption, total work and mechanical efficiency of the heart.

Failure of the left ventricle in this preparation was due to an increase in load, a decrease in contractile power or both of such a degree that the chamber began to fail to do the work imposed upon it by the load. This failure was characterized by a decrease in the ratio of both oxygen consumption and total work to diastolic volume and no change in mechanical efficiency.

The influence of follicular hormone on urinary secretion in man.

By B. G. SHAPIRO. (*From the Department of Clinical Endocrinology and the Physiological Laboratory, Guy's Hospital, London*)

v. Hann [1918] concluded, from a study of autopsy records, that the polyuria of diabetes insipidus could only occur in cases with posterior lobe deficiency provided the anterior lobe was still functioning. There is considerable evidence that œstrin can exert an inhibitory influence on the anterior lobe of the pituitary. If v. Hann's findings are correct then œstrin, by diminishing the activity of the anterior lobe, should lead to a diminution in urinary output. In the course of certain metabolic investigations in patients undergoing œstrin therapy, the opportunity arose to study the effect of œstrin on urinary output. The results were as follows:

SMALL DOSES OF ŒSTRIN

No effect on urinary output was observed with (*a*) daily injections of doses up to 7000 I.U. œstrone for 14 days, (*b*) courses of five injections of up to 50,000 I.B.U. œstradiol benzoate per injection, during 14 days.

LARGE DOSES OF ŒSTRIN

(*a*) In five patients with normal urinary output *daily* injections of œstradiol benzoate of 50,000–150,000 I.B.U. produced a decrease in urinary output. The lowest level reached was to 30 p.c. of the minimum pre-injection output for the individual.

(*b*) In two cases of proved diabetes insipidus the urinary output was reduced, the lowest level reached being 50 p.c. of the minimum pre-injection level.

The fact that œstrin diminished the urinary output not only in diabetes insipidus, but also in patients whose urinary output was normal, suggests that the anterior lobe is concerned both in the regulation of normal urinary secretion and in that of diabetes insipidus.

In one case of pituitary basophil adenoma which had undergone deep X-ray therapy, with resulting diminution in pituitary activity, the effect of large doses of œstrin was only very slight. It is possible that, since in this case the X-ray therapy had already diminished anterior lobe activity, the inhibitory influence of œstrin could not be so effective.

To test the assumption that the inhibitory effect of œstrin on urinary secretion is due to an inhibition of the anterior pituitary, the effect of anterior lobe preparations on urinary secretion in the human being is being investigated.

In certain cases the withdrawal of œstrin therapy leads to a "release" phenomenon, in which the urinary output greatly exceeds the pre-injection level. The degree of polyuria appears to be considerably more than a simple compensation for the preceding oliguria.

The use of œstrin to depress the urinary output in diabetes insipidus is not advocated for therapeutic purposes. Œstrin probably acts by depressing the anterior lobe, which is not clinically desirable.

I am indebted to Organon Ltd. for generous supplies of Menformon (œstrone) and Dimenformon (œstradiol benzoate).

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The production of antisera to prolactin. By F. G. Young

It was previously reported that an antiserum to prolactin was not obtained by methods which readily gave antisera to the thyrotropic and gonadotropic hormones of the anterior pituitary gland [Young, 1937]. Further experiments have shown that the daily administration of ox-prolactin to rabbits and monkeys for a very long time may produce potent antisera to prolactin, as demonstrated by the pigeon crop-gland test. In Table I the results are expressed in terms of the amount of serum required daily for 6 days to neutralize an amount of prolactin which will give an increase of 3 g. in the crop gland of an adult pigeon under the conditions of the pigeon test described by Rowlands [1937].

TABLE I

Donor animal	Period for which daily injections of prolactin were given weeks	Amount of serum required daily to prevent a crop growth of 3 g. c.c.
Rabbit	10	1·0
	13	0·5
	18	0·3
Monkey	10	1·0
	16	0·5
	38	0·2

It will be seen that a substantial anti-prolactin activity develops in the serum from rabbits and monkeys receiving daily injections of prolactin for 18 weeks or more. When 0·5 c.c. of potent anti-prolactin serum was injected daily into each of a series of lactating mice the total weight of litters reared was subnormal, presumably indicating a partial inhibition of milk secretion by the serum. Consistent results were not obtained when the sera were tested for ability to inhibit the glycotropic activity of prolactin in rabbits.

Control experiments using normal serum were carried out in all these assays.

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Antitrypsin and antithrombase. By J. MELLANBY and C. L. G. PRATT

Ox serum contains antithrombase and antitrypsin. Since thrombase may be a proteolytic enzyme, it is possible that antitrypsin and antithrombase are identical.

A series of experiments, however, showed that the antitryptic activity of serum is not diminished by the addition of sufficient thrombase to neutralize the antithrombase present, nor is the antithrombase activity decreased when trypsin is added to overcome the antitrypsin content of the serum. Further, the antitrypsin activity of serum is the same as the plasma from which it is obtained. Antitrypsin and antithrombase are therefore separate entities.

The experimental results also show that the antitrypsin of serum does not inactivate trypsinogen or enterokinase.

Mode of action of anticholinesterases on the central nervous system (spinal cord). By A. Schweitzer, Samson Wright (*Department of Physiology, Middlesex Hospital Medical School, London*), and Edgar Stedman (*Department of Medical Chemistry, Edinburgh*)

Schweitzer & Wright [1937] and Kremer *et al.* [1937] have shown that certain members of the anticholinesterase group of drugs exert a direct action on the spinal cord and influence transmission processes within it. Eserine increases reflex excitability and produces convulsions; prostigmine is a central depressant, diminishing or abolishing spinal reflexes. Acetylcholine injected intravenously (in the cat under chloralose anæsthesia) depresses spinal reflexes.

Employing our usual technique we have extended our observations and our results are summarized below:

Central convulsants	Central depressants
Eserine (44)	Eserine methiodide (45)
Dimethylcarbamic ester of hordenine hydrochloride (41)	Dimethylcarbamic ester of hordenine methiodide (42)
Stedman's "meta" hydrochloride	Stedman's "meta" methiodide
Miotine hydrochloride (28)	Miotine methiodide
Dimethylcarbamic ester of *m*-dimethylaminophenol hydrochloride (31)	Dimethylcarbamic ester of methyl hordenine methylsulphate
Dimethylcarbamic ester of 8-hydroxyquinoline hydrochloride (37)	Dimethylcarbamic ester of *m*-hydroxyphenyldiethylmethyl ammonium iodide
	Prostigmine
	Methylcarbamic ester of 3-oxyphenyltrimethyl ammonium methylsulphate (13)
	Methylphenylcarbamic ester of 3-oxyphenyltrimethyl ammonium methylsulphate (36)

(The numbers in brackets refer to Aeschlimann & Reinert's [1931] series.)

The central action of the anticholinesterases examined (whether convulsant or depressant) bears some relationship to their degree of anticholinesterase activity. The convulsant action of eserine is 50–100 times as great as that of substance 41, and Stedman has shown that its anticholinesterase activity *in vitro* is likewise about fifty times as great. Similarly, among the depressant compounds Stedman's "meta" methiodide is far more potent both in its action on the spinal cord and *in vitro* than prostigmine. Substance 41, when deprived of its urethane grouping, and thus of its anticholinesterase activity, loses its convulsant action.

Examination of the table shows that the depressant anti-cholinesterases have a structural resemblance to one another in respect of the fact that their basic nitrogen atoms are present in the form of quarternary ammonium groups. The convulsants investigated are without exception salts of tertiary bases. This difference in the nature of the basic group would presumably cause differences in the distribution of the drug in the tissues. The quarternary ammonium compounds are, as such, necessarily soluble in water and insoluble in lipoids. The salts of the tertiary bases, however, undergo some hydrolytic dissociation in solution, giving rise to an equilibrium mixture of basic ions and free base, and the latter will be soluble in lipoids. The difference in this repect (and perhaps also in their surface activity) may account for the different kind of action which these substances exert on the spinal centres.

In view of our findings it is not unreasonable to suggest that the central action of both convulsant and depressant anti-cholinesterases may be due to preserving naturally formed acetylcholine in the spinal cord. The difference in their action may depend on the site of their activity in relation to the surface membrane of the nerve cell or its related synaptic terminals ("neuropil"). Thus the quarternary lipoid-insoluble anticholinesterases may preserve acetylcholine outside these membranes, and so produce central inhibition. The tertiary anti-cholinesterases (which give rise to lipoid-soluble free base) may, on the other hand, preserve acetylcholine inside these membranes or modify the permeability of these membranes to acetylcholine; they may thus facilitate transmission processes or initiate a discharge of motor impulses from the spinal centres. On such a theory it would be possible for acetylcholine to act both as a central excitatory and inhibitory agent within the spinal cord; it is possible that the level of activity of anterior horn cells may depend on the relative concentration of acetylcholine within and without the cells.

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The effect of sound on the ear in reptiles. By E. D. Adrian

In 1930 Foa & Peroni published an investigation of the action currents in the auditory nerve, using the turtle *Thalassochelys* on account of the ease with which its nerve could be prepared. They found electrical activity in the nerve when the ear was exposed to sound, but the frequency of the potential oscillations always lay between 50 and 60 per sec. whatever note was sounded. In the same year Wever & Bray [1930] showed that in the mammalian ear the potential changes followed the frequency of the sound waves up to several thousand cycles per sec. Since their result seemed to contradict that of Foa & Peroni, Wever & Bray [1931] examined the response to sound in the painted terrapin, *Chrysemys picta*. Here they found potential changes in the nerve varying from 120 to 1000 cycles per sec. in agreement with the sound, though the ear was relatively insensitive to sounds above 500 cycles. They concluded that Foa & Peroni were wrong in thinking that the 8th nerve response had a fixed frequency but they could not explain the discrepancy in the results.

As a sequel to some class demonstrations on the frog, the reaction of several reptilian ears to sound has been examined and a possible explanation has appeared. In the common land tortoise and in the box tortoise (*Cistudo*) the ear is extremely sensitive, judged by the nerve response, to sounds over a very narrow frequency range. In all the specimens examined the optimum frequency has been about 110 cycles per sec. Sounds below 80 cycles and above 130 are usually without effect unless they are loud, whereas over the 105–115 range a distinct nerve response is produced by sounds only just audible to the human ear. The narrow range of high sensitivity is due either to a resonating mechanism or to the failure of the nerve fibres to respond at higher rates. The head of the columella forms a thin plate with its aboral margin fixed and its oral

margin free to move. This plate may tend to vibrate with a natural period of 110 per sec. although it must be damped by the thick membrana tympani [cf. de Burlet, 1934].

Over the range in which a response is obtained the frequency of the action potentials varies with that of the sound. But the range is small and in records of the response to impure sounds of varied pitch the critical frequency will predominate, giving the impression that the frequency is relatively constant whatever the sound. The range in Foa & Peroni's turtles was 50–60 per sec., only half that in the tortoise. This suggests a

Fig. 1. Action potentials in 8th nerve of tortoise in response to sound of increasing pitch (singing "ah"), showing narrow frequency range of response.

middle ear mechanism tuned to a lower value, though in some tortoises low tones have tended to give a response at half the critical frequency, i.e. at 50–60 per sec. In any case Foa & Peroni's observation, if due to such a limited range of sensitivity, does not conflict with the mammalian results. In the terrapin, presumably, a sharp tuning does not occur.

In the alligator, which has a membranous ear drum and a true cochlea, the frequency of the electric response follows that of the sound up to 1000 cycles or more. The sensitivity, judged by the nerve response, falls off rapidly at high frequencies. In the alligator, as in birds and mammals, electrical oscillations corresponding more or less to the sounds can be picked up from any part of the inner ear and there is some spread of the cochlear potentials into the 8th nerve. In the tortoise this Wever & Bray effect is absent and the currents in the nerve are due entirely to action potentials in its fibres.

In the grass snake loud sounds failed to produce any sign of activity in the 8th nerve. Tactile stimuli and vibration of the supporting surface gave an irregular discharge.

Opinion seems to be divided as to whether tortoises can hear [cf. Gadow, 1909; Kuroda, 1925-26]. It must be admitted, however, that the tortoises so far examined have shown no sign of appreciating the sounds to which their ears are sensitive.

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Maintenance and prolongation of pregnancy in hypophysectomized rabbits by œstrogenic substances. By J. M. Robson

It has previously been shown [Robson, 1937] that when rabbits are hypophysectomized during pseudo-pregnancy the luteal activity can be maintained by the administration of œstrogenic substances. The present experiments were made to determine whether the luteal function could be maintained by the administration of œstrin in animals hypophysectomized during pregnancy.

The pituitary was removed in rabbits 21 days after mating and daily doses of 3–10 μg. of œstrone or 5 μg. of œstradiol (dissolved in oil) were then given. In all experiments the abortion, which otherwise occurs within 24–48 hours, was prevented. Live fœtuses were present in the uterus 6 days after hypophysectomy in only a minority of the experiments; in the others the fœtuses were dead and reabsorption was beginning. In all the animals the corpora lutea were large and histologically like those of normal animals at the same stage of pregnancy.

In another series of experiments hypophysectomy was performed on the 28th or 29th day after mating and daily doses of 10 μg. of œstrone or 5 μg. of œstradiol were then given. Parturition did not occur and the pregnancy was prolonged in all the experiments; in some experiments fœtuses were expelled 4–6 days after the normal time. The corpora lutea were large and histologically similar to those seen towards the end of a normal pregnancy.

The daily administration of 40 mg. of the synthetic œstrogenic substance, triphenyl ethylene [Robson & Schönberg, 1937], to rabbits hypophysectomized on the 21st or 29th day of pregnancy maintained or prolonged the gestation in the same way as did œstrone and œstradiol.

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Cyclical fluctuations in the sensitivity of the rat to œstrogenic stimulation. By S. ZUCKERMAN

The sensitivity of the uterus of spayed rhesus monkeys to œstrogenic stimulation appears to vary cyclically [Zuckerman, 1937*a*, *b*]. The observations on which this conclusion rests have been multiplied, and they suggest that some extra-ovarian factor which affects the uterus directly needs to be taken into account in the explanation of rhythmical changes in the accessory reproductive organs of these animals. This generalization would appear to apply to the rat also. Thus Castillo & Calatroni [1930] report that œstrus recurred periodically in a group of spayed rats which were injected daily with a constant low dose of œstrin. I have confirmed this observation in experiments carried out on twenty-three animals. The individual variation in the level of œstrogenic stimulation at which these cycles appear is from 5 to 15 I.U. of œstrone (given subcutaneously in oil solution at a concentration of 10γ per c.c.), the mean being 8·9 I.U. If too much œstrone is given, continuous œstrus is produced; if too little, phases of prolonged anœstrus occur. The modal and maximum duration of œstrus in normal rats are 27 and 81 hr. respectively [Long & Evans, 1922]. Since the animals used in the present experiments were smeared only once daily, cycles in which œstrus lasted for 4 days or more have been taken to indicate that too much œstrone was being given, and have been left out of the calculations. Cycles of 10 days or of longer duration were regarded as being phases of anœstrus, and have also been excluded. Such cycles occurred in only 6 p.c. of Long & Evans's series of 1999 observations, and the chance of being in error in leaving them out is less than 1 in 10.

A total of 203 successive "artificial œstrous cycles" were recorded in the twenty-three rats investigated. The mean cycle length was 5·3 days. The mean duration of the normal œstrous cycle of the rat is 5·4 days [Long & Evans]. As in the normal animal, too, the most frequent cycle lengths were 4, 5 and 6 days.

It should be noted that whereas the occurrence of periodic uterine bleeding in spayed monkeys that are injected daily with a constant threshold dose of œstrone indicates the periodic raising of the œstrin threshold, the occurrence of periodic œstrus in spayed rats that are treated in the same way indicates the periodic lowering of the threshold.

Two further observations which indicate that the accessory reproductive organs of female mammals are affected cyclically and directly by

some extra-ovarian factor may be noted. Kostitch & Telebakovitch [1929] report that a vaginal cycle of epithelial changes occurs in spayed as well as in normal mice, a preponderance of mucus, leucocytes, and nucleated epithelial cells following each other in regular order. Stockard & Papanicolaou [1917] report that the degeneration of the uterine and vaginal mucosa in the guinea-pig after ovariectomy may occur in periodic bursts.

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The adrenals and pituitary in relation to cyclical changes in the œstrin-sensitivity of the rat. By S. Zuckerman

Experiments carried out on eight spayed rats show that threshold "artificial œstrous cycles" [Zuckerman, 1938] will still occur after hypophysectomy. The cycle does not alter significantly in length, but the threshold level at which it shows itself is consistently lower than in animals that are only spayed (a mean of 4 I.U. daily as compared with a mean of 8·9 I.U.). Five of eight other rats in whom artificial cycles had previously been established went into, and remained in, œstrus during the survival period following the operation, the injections being continued at the same level as before.

Observations on the relation of changes in water-metabolism to œstrogenic stimulation [Krohn & Zuckerman, 1937; Guthkelch & Zuckerman, 1937; Thorn & Harrop, 1937] suggest that the extra-ovarian factor which cyclically alters the œstrin-threshold of the accessory reproductive organs may be the adrenal cortex [Long & Zuckerman, 1937]. If this hypothesis is valid, it should be impossible to elicit artificial œstrous cycles in spayed and adrenalectomized rats that are injected daily with constant doses of both adrenal cortical hormone and œstrone.

The daily amount of cortin (Upjohn Company) that is necessary to maintain the normal cycle in rats deprived of their adrenals was determined in a preliminary set of experiments on six animals. Seven

spayed rats in which a series of successive artificial cycles had been established were then adrenalectomized and given this amount of cortin daily, œstrone being administered as before. Cycles have continued in only three of the animals (still under experiment), but each cycle has been prolonged. Three hypophysectomized and spayed animals in which cycles had previously been established were also adrenalectomized and injected in the same way with cortin and œstrone. Attempts to continue the cycles were unsuccessful, anœstrus or phases of prolonged œstrus occupying the survival period after the operation (up to 24 days).

These experiments are being continued, but it may already be concluded that, if an endocrine mechanism is implicated in the occurrence of cyclical changes in the sensitivity of the rat to œstrogenic stimulation, the adrenal cortex and not the pituitary is the gland likely to be concerned.

I wish to express my best thanks to Dr R. L. Noble for his kindness in performing the hypophysectomies.

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Metabolism of the dog made diabetic by anterior pituitary injections. By H. P. Marks and F. G. Young

The urinary excretion of glucose, nitrogen and ketones has been studied in two of the dogs rendered permanently diabetic by injection of anterior pituitary extracts. In one of them the influence of extensive variations in the type of diet has also been investigated. On a diet of lean meat and boiled liver, and consisting, therefore, essentially of protein, the corrected average D/N ratio was about 3 : 1, probably indicating maximal formation of sugar from protein. On this diet extra glucose given by mouth appeared to be almost quantitatively excreted.

When, however, a large proportion of the protein was replaced by equicaloric amounts of preformed carbohydrate, the sugar excretion was decidedly less than the amount to be expected, from the preformed carbohydrate together with the ingested protein.

On a diet consisting almost entirely of fat, the glycosuria of the one dog examined was practically abolished and ketonuria definitely diminished.

Dogs made permanently diabetic by anterior pituitary injections. By F. G. YOUNG

If dogs are given daily injections of a crude extract of fresh ox anterior pituitary lobe for 2–4 weeks, the diabetic condition which results from such treatment may persist after cessation of injections, and become permanent [Young, 1937]. In six experiments in which suitable large doses of extract were administered there has been only one failure (already reported [Young, 1937]) to produce a permanently diabetic condition in this way.

The diabetic condition so produced differs in some respects from that of depancreatized dogs, most strikingly in that the animals made diabetic by pituitary injections are able to survive without insulin, retaining good vigour without any very great loss of body weight. Nevertheless, the severity of the diabetic condition of these dogs appears to be comparable with that of depancreatized animals.

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The effect of androgenic substances on the testes and accessory reproductive organs of the immature male guinea-pig. By A. C. BOTTOMLEY and S. J. FOLLEY. (*National Institute for Research in Dairying, University of Reading*)

It is well known that administration of œstrogenic hormones causes inhibition of the gonadotrophic activity of the anterior pituitary accompanied under some conditions by pituitary enlargement. Observations by a number of workers are best explained by assuming that androgenic substances exert a similar action. The following results indicate the striking effect of crystalline androgenic substances in causing atrophy of the testes in the young guinea-pig and give some idea of the relative effectiveness of a series of related substances.

Each of the androgenic substances named in Table I was administered to a group of five immature male guinea-pigs weighing about 175 g., subcutaneous injections of 2 mg. androgen in sesame oil being given daily for about 30 days, except in the case of testosterone propionate where the equivalent of 2 mg. testosterone was given daily. Where possible the daily dose of oil was 0·2 ml., but in the case of the less soluble diols the daily

oil volume was 0·8 ml. At the end of the injection period, which varied slightly from group to group, the animals were killed and the relevant organs weighed.

TABLE I. Mean weight (mg.) per 100 g. body weight

Injection	Testes	Seminal vesicles	Prostate
(A) 0·2 ml. solution injected daily			
Sesame oil (control)	279·9	100·9	49·7
Androstenedione	88·9	154·8	117·4
Androsterone	193·3	142·5	68·6
trans-Dehydroandrosterone	258·4	142·2	71·3
Dihydrotestosterone	49·9	204·4	100·8
17-Methyltestosterone	45·2	237·3	136·4
Testosterone propionate	74·9	306·7	99·1
(B) 0·8 ml. solution injected daily			
Sesame oil (control)	198·3	122·7	64·3
Androstanediol	37·3	145·0	63·7
trans-Androstenediol	74·4	64·5	54·6
Testosterone	52·7	114·0	86·5
Testosterone propionate	71·4	291·0	125·6

It will be seen that all the androgens used, except androsterone and *trans*-dehydroandrosterone, caused a marked regression of the testes, the difference from the control value in each case proving highly significant on application of the usual statistical test. The size of the accessory organs is of course directly influenced by the injections and does not indicate the functional activity of the testes.

In view of the relationship between the anterior pituitary and the gonads it is reasonable to assume that the regression of the testes observed in these experiments was due to suppression of the anterior pituitary by the action of the androgenic substances.

Experiments on the coincident injection of gonadotropic extracts, as well as histological examination of the hypophyses and thyroids, are being undertaken with a view to testing this supposition.

We are grateful to Dr K. Miescher and Messrs Ciba, Ltd., for the gift of the androgens used in this work and to Mr J. McClemont for the provision and care of the guinea-pigs.

The inhibitory action of acetylcholine on water-diuresis in the dog. By M. PICKFORD[1]

Dikshit [1934, 1935] showed that acetylcholine injected into the hypothalamus and into the cerebral ventricles produced effects similar to those of electric stimulation in those parts, and Chang *et al.* [1937] showed that a reflex secretion of a pressor substance of posterior pituitary

origin could be elicited by the central stimulation of the vagus nerve. It seemed possible, therefore, that acetylcholine might play a part in the liberation of the posterior pituitary hormones, and that this might be demonstrated by the consequent inhibition of water-diuresis.

Bitches were used and water-diuresis induced by giving 300 or 350 c.c. of water by stomach-tube. The urine was collected by means of a self-retaining catheter inserted in the bladder. At the time of giving the water 1 mg. of atropine sulphate was injected intravenously to counteract the peripheral effects of acetylcholine. When the diuresis was established 0·2 mg. acetylcholine in 0·2 c.c. 5 p.c. acid sodium phosphate was injected intravenously. The result was no appreciable disturbance of the animal, but a marked inhibition of the water-diuresis, the form and course of the inhibition resembling closely that due to a small quantity of posterior pituitary extract and not being at all like the inhibition produced by an intravenous injection of adrenaline.

When 1 mg. of acetylcholine was given there was complete anuria for 7–8 min., after which the rate of excretion steadily recovered. With doses of this size there was also momentary panting and muscular weakness, and the mucous membranes were blanched. The normal colour of the membranes returned 30 min. before the urine flow reattained its maximum rate. With 0·2 mg. acetylcholine there was no noticeable pallor of the membranes.

Further work is in progress to elucidate the mechanism of this inhibition of water-diuresis by acetylcholine. The observations so far made do suggest that the inhibition may be due to a release of posterior pituitary hormone.

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Effect of preganglionic nerve section on ganglionic transmission.

By Z. M. Bacq and G. Coppée. (*Institut Leon Fredericq, Liége*)

It has been reported by Titeca [1935] that the section of a cat's motor nerve is followed within 30 hr. by a neuro-muscular block for which the Wallerian degeneration is not responsible, since almost unimpaired action potentials can be detected in the nerve trunk. We have

observed a similar block in the transmission of nerve impulses across the synapses of the superior cervical ganglion of the cat.

The preganglionic fibres were divided by cutting the cervical sympathetic nerve at the base of the neck. 50–70 hr. after the section, electrical stimulation of the peripheral end of the cut nerve no longer produces a contraction of the nictitating membrane. The preganglionic fibres are not yet degenerated, however, since action potentials are still detected in the nerve trunk; and the impulses reach the ganglion, as shown by a diphasic record when the "grid" lead of the amplifier is placed on the intact ganglion.

The block is preceded by increased duration of the contractile responses of the membrane to short groups of preganglionic volleys. This is due to the well-known sensitization of the membrane to "sympathin" or adrenaline. At this stage single stimuli are ineffective and prolonged stimulation shows a fatigue phenomenon. This fatigue is located neither in the plain muscle nor in the preganglionic nerve fibres, since the action potentials do not decay during prolonged stimulation. The fatigue seems to be located in the ganglion synapses, like the block of transmission which it precedes. MacIntosh (see next communication) has observed that acetylcholine disappears from the ganglion simultaneously with this early impairment and failure of transmission.

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Biological properties of gonadotrophic extract of pregnant mare serum. By I. W. Rowlands. (*From the National Institute for Medical Research, Hampstead*)

Observations have been made on the biological properties of a series of highly purified extracts of pregnant mare serum, assayed by different methods. The tests used were designed to show the capacity of the extracts to cause (*a*) follicle stimulation, (*b*) ovulation, (*c*) corpus luteum formation, and (*d*) œstrogen production by the ovary. The following criteria were used:

(1) Development of the ovary of the immature rat.
(2) Follicular maturation in the ovary of the anœstrous ferret.
(3) Growth in the uterus of the immature guinea-pig and rabbit.
(4) Ovulation in the œstrous rabbit.

The response of the ovary of the immature rat is not limited in the same way as with extracts of urine of pregnancy and of human pregnancy serum [Boycott & Rowlands, 1938]. The maximum weight of ovary obtained with these latter preparations is about 50 mg. whereas ovaries weighing over 200 mg. can be obtained with extracts of pregnant mare serum. The gonadotrophic properties of extracts of pregnant mare serum are therefore different from those of human pregnancy serum, and, by this test, resemble those of pituitary extracts. A close correlation was found between the relative activity of the extracts on the immature rat and the labelled activity of the preparations in mouse units.

Experiments on anœstrous ferrets showed that the extracts cause rapid development of quiescent follicles, and, with adjustment of the dosage, an almost pure follicle-stimulating effect without luteinization. It has not been found possible so far to produce ovulation or corpus luteum formation in the anœstrous ferret with these extracts, but the thecal luteinization which occurs when larger amounts are injected suggests the presence of a small amount of luteinizing substance in the extracts. The relative activity of the extracts when tested for follicle development in the ferret is in agreement with their activity on the immature rat or mouse.

The extracts caused comparatively little increase in ovarian weight in the immature guinea-pig or rabbit, but great enlargement of the uterus was produced, indicating a plentiful secretion of œstrogen by the ovary. No uterine growth was obtained in the ovariectomized animal.

The relative amounts of the various extracts required to produce ovulation of the mature follicles present in the ovary of the œstrous rabbit are somewhat different, although a general relationship exists between ovulation-producing and follicle-stimulating capacity.

These extracts of pregnant mare serum had a wide range of quantitative activity (2500–30,000 mouse units per g., some of them being as much as 99 p.c. diluent), but the various biological tests carried out have not yielded definite evidence of qualitative difference in preparations from this source.

The extracts of pregnant mare serum ("Antex") were generously made available by Mr A. Kongsted, Lovens Kemiske Fabrik, Copenhagen, to whom best thanks are due.

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The reversal of neuro-muscular block by catelectrotonus. By BERNHARD KATZ. (*Department of Physiology, University College, London*)

Nerve-sartorius preparations of *Rana esculenta* were used, at 16–19° C., nerve and muscle being stimulated by separate pairs of electrodes (Ag- or Pt-wires for nerve, fluid electrodes and calomel half cells for muscle).

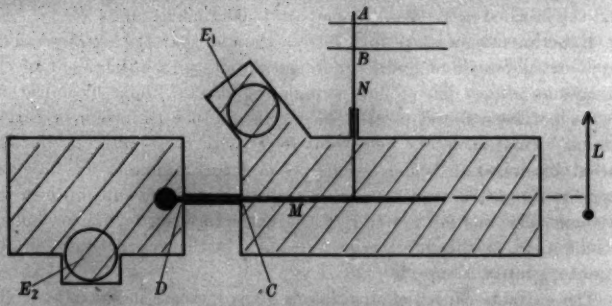

Fig. 1. Paraffin block, containing fluid bath with nerve-muscle preparation. *N*, nerve; *M*, sartorius muscle, seen from edge; *A*, *B*, electrodes on nerve; *C*, *D*, points of current entry in muscle; E_1, E_2, contact between fluid bath and calomel half cells; *L*, tension lever (optical or kymograph).

Fig. 2. Curarization by large excess of $CaCl_2$ (8–9 times normal, as used in the majority of the experiments). Response of sartorius to nerve stimulation, alone (*a*), and with previous application of subthreshold constant current to muscle (*a*, anode at *C* (Fig. 1); *c*, cathode at *C*). Time marks: 1 sec.

When a weak neuro-muscular block has been produced (by curarine, strychnine, Mg, large excess of Ca, fatigue), transmission across many myoneural junctions can be restored (i) by sending in two nerve volleys following one another at a short interval [cf. Adrian & Lucas, 1912; Bremer, 1927], (ii) by applying, in addition to a single nerve volley, a subthreshold constant current to the muscle with the cathode near the myoneural region.

(*a*) With increasing time interval between break of a cathodal pulse to the muscle (0·25 sec. duration) and arrival of a nerve impulse at the myoneural junction, the "excess response", due to the facilitating effect of the constant current pulse, disappears gradually (falling to 1/2, on the average, in 15–20 msec., a small remainder, however, persisting often with intervals $>1/10$ sec.). The "excess response" diminishes appreciably if the nerve impulse arrives (allowing for conduction time) less than 5–6 msec. before the break of the current pulse. Apparently the local excitatory process in the muscle fibre, produced by the arriving impulse, requires several msec. to reach its maximum.

(*b*) When two nerve impulses follow one another, the facilitated response of the muscle rises sharply, as the interval increases, passes through a maximum at about 7 msec., and then falls gradually. Comparing (*a*) and (*b*), the falling curves (plotting "excess response" against time interval) are nearly the same, whether facilitation is by a preceding nerve impulse or by a cathodal pulse to the muscle.

(*c*) A similar curve, though often declining somewhat more rapidly, is obtained when the subthreshold current pulse is followed by a brief testing shock, both stimuli being applied directly to the muscle (more completely curarized, or with cathode on pelvic end). This relation, falling again during about 0·1 sec., corresponds to the slowly subsiding "α-excitability" of muscle [Rushton, 1931].

It appears that the local excitatory process in muscle, left behind by a "subliminal" nerve impulse, has a protracted phase (period of "facilitation", or "supernormal conductivity", Adrian & Lucas [1912]), which can be imitated closely by application of an electric stimulus directly to the muscle, and the rate of decay of which may be related to that of the "α-excitability" of muscle.

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Effect of preganglionic section on acetylcholine in the ganglion. By F. C. MacIntosh. (*National Institute for Medical Research, Hampstead, London, N.W.* 3)

Brown & Feldberg [1936] have shown that, after section of the cervical sympathetic trunk in the cat, the normally high acetylcholine equivalent of the superior cervical ganglion falls to 10–20 p.c. of its original value. In view of Bacq & Coppée's [1938] finding that the ganglion becomes irresponsive to stimulation of the degenerating preganglionic fibres while conduction in the latter is still unimpaired, it seemed desirable to know how soon after section of the trunk the loss of ACh. from the ganglion takes place.

The preganglionic trunk of one side was cut aseptically in ten cats; after varying intervals the ACh. extractable by trichloroacetic acid from the normal and from the decentralized ganglion was assayed on the eserinized leech muscle and on the blood pressure of the cat. 24 hr. after section, the decentralized ganglion contained on the average (four animals) 15 p.c. less ACh. than the control ganglion; 48 hr. after section (two animals), 60 p.c. less; and 72 hr. after section (four animals), 70 p.c. less. The peripheral response (pupil and nictitating membrane) to stimulation of the degenerating preganglionic trunk was reduced after 48 hr. and absent after 72 hr.

Thus, the interval between section of the preganglionic fibres and failure of the ganglion to transmit impulses, corresponds to that during which the ganglion loses most of its preformed ACh.

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The preparation of the Tibialis anterior (cat) for close arterial injections. By G. L. Brown

The preparation of gastrocnemius and soleus for close arterial injection has already been described. Tibialis anterior offers many advantages over gastrocnemius on account of ease and speed of preparation, tensions more convenient for record, uniform composition of pale fibres and long tendon, making it suitable for electrical recording.

In decerebrate preparations, the leg is first immobilized by section of the sciatic, hamstring and femoral nerves and the tendon of iliopsoas.

The popliteal space is opened and the tibial nerve crushed or severed. The tendon of tibialis is then freed from its attachment, together with a fragment of bone, and the skin incision is extended over tibialis to the head of the tibia. The transverse ligament of the ankle is cut and the muscle is freed and raised from the underlying Extensor longus digitorum. The anterior tibial artery can then be seen lying posterior to the belly of Tibialis anterior; it is freed just proximal to the ankle joint, the fine vessels passing to Extensor longus digitorum are tied, and the latter muscle is removed. A second layer of muscles is then exposed—the peroneal group—and the branches passing to them from the anterior tibial vessels are also tied. The artery can now be followed to the point where, after penetrating the interosseous membrane, it passes through the origin of the Tibialis anterior, and a thick thread is passed under it and loosely knotted. The animal is now fixed to the myographic stand by drills through the lower end of the tibia, the condyles of the femur, and, occasionally, also through the upper end of the femur, and the tendon is attached to the myograph lever. The anterior tibial artery is tied near the ankle joint, a bulldog clip is applied and a hypodermic needle cannula is inserted into the proximal end of the vessel and firmly clamped to the stand. Injections are made by attaching a syringe filled with 0·25 c.c. of fluid to the cannula, closing the tibial artery by traction on the thread, removing the clip and emptying the syringe into the muscle. The preparation gains by the fact that no large muscular areas are exposed, and the Tibialis anterior itself can conveniently be covered by suturing the skin until only the tendon is exposed, the cannula and artery still remaining easily accessible.

Effect of small doses of curarine on neuro-muscular conduction.
By G. L. Brown

Two maximal motor nerve volleys are set up at varying time intervals in a decerebrate cat, which has received a dose of curarine insufficient completely to block neuro-muscular conduction. As soon as the muscle action potentials are sufficiently far apart to be measurable independently, it is found that the second is some 10 p.c. larger than the first. This probably represents the facilitation phenomenon described by Bremer for partially curarized frog muscle. As the volleys are separated in time, the disparity lessens, and at about 50 msec. the two are equal. Further separation of the volleys reveals an increasing depression of the response

to the second volley, which reaches its maximum between 200 and 400 msec., at which point the second volley may evoke a response only 50 p.c. of the first. From this point the second response gradually increases in size but is still less than the first at an interval of 1 sec.

The results indicate that there is a long lasting depression following a first response, and that this, in its earlier phases, is more than counterbalanced by a facilitation due to a different process. This is further exemplified by the effects of short tetani. A tetanus at 50 per sec. for 0·5 sec. produces an increase in the size of action potential and tension in a subsequent single twitch, presumably on account of summation of the facilitations and the recruitment of additional muscle fibres. On the other hand, a tetanus of the same duration, but consisting of only five stimuli, is followed by reduction in the size of action potential and tension of a following twitch.

The long depression causes, during a tetanus, a progressive reduction of action potentials to a minimum short of extinction. Thus, during the first ten responses to stimulation at 50 per sec. the potentials show a steep, exponential decline to 25 p.c. of the initial value, at which they remain while the tetanus lasts.

It is suggested that the long depression is due to insufficiently rapid replacement of acetylcholine, to which curare has raised the threshold of the muscle fibres. The initial facilitation is a separate phenomenon.

Neuro-muscular conduction in the fowl.

By G. L. Brown and A. M. Harvey

Optical isometric myograms and action potential records have been taken from the lateral head of the gastrocnemius of the fowl anæsthetized with pernocton. Slightly supramaximal break shocks were applied to the nerve twig supplying the muscle.

The initial tension of the muscle has a very striking effect upon the twitch tension. Thus, with no initial tension, the twitch tension was 170 g. Increase in initial tension up to 130 g. produced a twitch tension of 540 g. The action potentials, simultaneously recorded, showed random variations of less than 5 p.c.

When two volleys are set up in the nerve, less than 200 msec. apart, the action potential in response to the second is regularly greater than the first. When the second response is measurable, i.e. at 20 msec. interval, it may be 25 p.c. greater than the first. As the volleys are

separated, the second response progressively diminishes in size until the two are equal. If the two volleys are set up shortly after a brief tetanus, the first response is either unaltered or a little increased, but the second becomes as much as 12 p.c. smaller than the first. The higher the frequency of the preceding tetanus the greater the relative depression of the second response.

The time course of the increase in the second response is closely similar to that observed in mammalian muscle under weak curare [Brown, 1938] and suggests that in fowl muscle a single nerve volley does not excite all the muscle fibres and that a process of facilitation persists from the first response, allowing the second volley to excite additional fibres. "Staircase" appears to be excluded by the regularity of the phenomenon, the restriction of the effect to the first two responses of a tetanus and the frequent absence of effect of a tetanus on the first of two subsequent responses. It must be pointed out that, in all these experiments, the muscle was at a considerably lower temperature than the normal.

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The permeability of the erythrocyte to cations.

By J. F. Danielli and H. Davson

A general investigation has been made of the conditions under which the erythrocyte becomes permeable to potassium. Centrifuging cells from Ringer solution causes significant losses probably due to a mechanical stretching of the cell membrane (ox, dogfish, rabbit, human, horse). Fluoride, cyanide, carbon monoxide, urethane and phenylurethane cause no appreciable loss at 20° C. At 37° C. fluoride causes an observable loss of potassium, confirming observations of Dr W. Wilbrandt. If the maintenance of the high potassium content were due to an active secretory process some of these substances should cause leakage of potassium. On the other hand pyocyanine and methylene blue might raise the potassium content of the cells. No such effect was observed with either nucleated or non-nucleated cells. The possibility that the permeability of the membrane to hæmoglobin produced by hæmolytic agents is preceded by a stage of permeability to cations was considered, and shown to be the case with heavy metals of which silver, which may cause leakage in $1 \cdot 10^{-6}$ *M* concentration, has been particularly studied; saponin does not show this effect.

Effect of temperature on the response of the gut to adrenaline. By H. BLASCHKO and H. SCHLOSSMANN. (*From the Physiological Laboratory, Cambridge*)

The response of the rabbit's intestine to small doses of adrenaline is more marked at a temperature of about 30° than at body temperature.

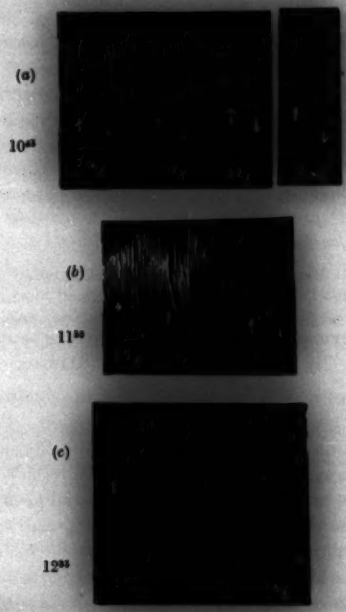

Fig. 1. Rabbit's intestine at (*a*) 28·5°, (*b*) 38·5° and again at (*c*) 28·5°. Varying amounts of adrenaline added to 60 c.c. of Tyrode solution (↑). ↓ = adrenaline washed out. Time in minutes.

This observation has proved useful for the biological assay of solutions containing adrenaline. Fig. 1 shows that the minimum amounts of adrenaline which come within the range of evaluation are smaller at 28·5° than at 38·5°.

Response of fowl to massive dosage of œstrone and testosterone.
By C. W. Emmens. (*National Institute for Medical Research, London*)

Full-sized combs were produced in capons by Freud *et al.* [1932] after 4 months' injection of a bull testis concentrate, and by Callow & Parkes [1935], in the course of a few weeks, using solutions of crystalline androsterone. The maximum rate of growth possible in the capon comb has now been determined by the implantation into the breast muscle of solid tablets of testosterone propionate. Brown Leghorn capons each received a single tablet of from 5 to 20 mg. The combs all responded at the same rate and grew to full size in 5 weeks; they maintained the growth for about an equal period, and commenced to atrophy again at 9–10 weeks after implantation. Scarcely any increase in growth rate is obtained by administering very much larger doses.

The combs of normal Brown Leghorn hens treated with injections of 25 mg. per week of testosterone propionate in oil solution have responded with somewhat smaller growth rates, but three at present under treatment give promise of producing combs little smaller, if at all, than those of normal cockerels. The amount of hormone needed to produce this growth is, however, much greater than that required by capons.

Depression of the gonadotrophic activity of the pituitary, following administration of large amounts of sex hormone, has been studied by many workers during recent years. The cock seems to be a particularly favourable object for investigating this action of œstrogens, since regression of the testis is accompanied by comb atrophy. Adult Brown Leghorn cockerels received a series of injections of œstradiol benzoate in oil; in the early stages fortnightly treatment with 10 mg. of the hormone proved effective, and later the same amount weekly. The comb atrophies almost to the level of a caponized bird within 2 months, but evidence has been found of a refractory condition after that period. On discontinuing the injections the comb grows again to its former size within a few weeks. The testes of a cock killed when the comb had reached a minimum size were very small, having regressed as much as do those of a bird hypophysectomized for a period of time equal to that covered by the injections. The thyroids were enlarged, but histologically showed inactive epithelium and great distention of all the follicles with colloid. Administration to one of the experimental birds of 5 mg. of testosterone propionate, together with double the usual dose of œstradiol benzoate (20 mg.), was followed by comb growth. This growth was,

however, less than that normally occurring after injection of such an amount of male hormone, and indicates that in this bird the comb atrophy obtained by injection of œstradiol was not due solely to pituitary depression. The extreme hypoplasia of the testis and thyroid in a similar bird, however, leaves no doubt that there is severe depression of the gonadotrophic and thyrotrophic activity of the pituitary.

Implantation of tablets of 40–100 mg. of œstrone has proved more effective than injections, but refractoriness is again observed after about 6 weeks.

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"Adrenoxine"; its production from adrenaline and its action.

By Z. M. Bacq. (*Institut Leon Fredericq, Liége*)

When adrenaline in dilute solution ($1 \cdot 10^{-5}$–$1 \cdot 10^{-7}$) is oxidized at neutral *p*H by a small amount of tyrosinase (from the mushroom or the meal-worm), or of catecholoxidase (from the potato), and tested on the blood pressure of a spinal or anæsthetized cat, the following facts are regularly observed [Heirman, 1937].

(1) In a few minutes the normal action of adrenaline disappears.

(2) The red solution, containing the adrenochrome of Green & Richter [1937], remains inactive for a long time (45–120 min.).

(3) There appears suddenly a powerful vaso-dilator action which persists for a very long time (4–8 hr. at laboratory temperature).

(4) The vaso-dilator action disappears slowly.

(5) Melanine precipitates in the solution.

Thus, between adrenochrome and melanine there is formed *in very dilute solutions only* an inhibitory substance which we propose to call "adrenoxine".

Such a vaso-dilator action appears if dilute solutions of tyramine, p. sympatol, epinine and oxytyramine are oxidized by tyrosinase. Oxidation of arterenol does not result in the production of an inhibitory substance.

The action of adrenoxine is not affected by ergotamine, piperidinomethylbenzodioxane (933 F.), or atropine, and is slightly increased by cocaine.

If an isolated frog's heart (Straub's technique) is treated with a purified preparation of mushroom tyrosinase, the organ responds by a

marked inhibition of its automatic contractions to sympathetic nerve stimulation [Bacq, 1937].

These facts show that adrenaline inhibition could be explained by the presence of a catecholoxidase in the inhibited structures. Investigations by Heirman and myself have revealed the existence of a catecholoxidase in the extracts of all smooth muscles inhibited by adrenaline, with the sole but constant exception of the intestine. Thus dilute extracts (10 c.c. of water for 0·1–3 g. of tissue) from bronchi, stomach and bladder (fundus only) from man, dog, cat, pig, sheep, rabbit, ox, guinea-pig, ferret, rat and frog, extracts of the coronary arteries of man and ox, and of the non-pregnant uterus of the cat, sheep and ferret are capable of changing more or less rapidly (45 min. to 4 hr.) adrenaline into adrenoxine. Similar extracts of intestine, and of many tissues giving only an augmentor or no response to adrenaline (liver included), do not inactivate dilute solutions of adrenaline.

The extracts of virgin cat's uterus and of frog's bladder and stomach oxidize catechol, pyrogallol and adrenaline to melanine. They are inactivated by heating for a few minutes at 46° C. In the presence of resorcinol *M*/500, or of a small concentration ($1 \cdot 10^{-5}$) of progesterone, the phenolasic action of the extracts is inhibited. I used a crystalline synthetic preparation kindly given by Messrs Schering-Kahlbaum. This effect of progesterone gives the probable explanation of the well-known reversal of the response of the cat's uterus to adrenaline, during pregnancy or after injection of progesterone.

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Effects of oxygen pressure as influenced by external temperature, hormones and drugs. By J. Argyll Campbell

Increase of oxygen pressure. Previously [Campbell, 1937] it was demonstrated that various factors (hormones, drugs, etc.) which increase cell oxidation enhance poisoning by oxygen at high pressures, viz. 6 atmospheres for 30 min. (Messrs Siebe Gorman's pressure chamber). It was also shown that thyroidectomy has a definitely protective effect against this oxygen poisoning in white rats with the external temperature

at 33° C.; the normal controls die under these conditions, but survive when the external temperature is about 24° C.

Since the pituitary gland exerts a thyrotropic influence the effect of hypophysectomy was tested under the above conditions and was found to have a similar—but less marked—protective influence. Dr I. W. Rowlands kindly removed the pituitary glands by Dr Selye's technique, and eight rats which eventually recovered completely from the operation were exposed to 6 atmospheres of oxygen for 30 min. at 33° C. Five of them survived the exposure, but within the next 4 days they died from after-effects. The protection was thus not so lasting as after thyroidectomy which afforded complete protection under the same conditions.

Decrease of oxygen pressure. Similar experiments are being performed with white rats (80–100 g.) exposed to low oxygen pressure. The same chamber is used but the barometric pressure is lowered to 240 mm. Hg for 32 min. and the oxygen in the inspired air is only about 7 p.c. atmosphere. When the external temperature is kept at about 20–25° C. all the rats survive, but if the external temperature is kept about 33° C. most of the rats die; Gelhorn [1937] has observed a similar result for temperature with mice and rats.

Thyroidectomy, we have now found, neither enhances nor relieves the effects of lowered oxygen pressure. Subcutaneous injections of thyroxine (0·4 mg. daily for a few days) enhance adverse effects of acute oxygen want, and so does a single injection of dinitrophenol (1·5 mg.), but frequent injections (daily) of pituitary extract have no definite effect one way or the other. These results therefore differ in some respects from, but in others resemble, those for increase of oxygen pressure [Campbell, 1937].

Gelhorn [1937] found in acute experiments with rats and mice that breathing carbon dioxide at 3 p.c. has some protective influence against lowered oxygen pressure; he found that both lowered oxygen pressure and breathing carbon dioxide cause a fall of body temperature which protects the animal to some degree. In a resting animal, decrease of alkalinity of blood lowers cell metabolism [Campbell, 1923], which may explain the temporary protective action of carbon dioxide during oxygen want. Under high oxygen pressure, carbon dioxide enhances the poisonous effects of the oxygen [Campbell, 1937].

The results for short acute experiments differ from those for experiments of a prolonged nature in which the animal is gradually acclimatized to lowered oxygen pressure. Thus in rabbits breathing about 5 p.c. carbon dioxide continuously for several weeks the powers of acclimatiza-

tion to lowered oxygen pressure are not increased. Rabbits are still unable to become acclimatized to 10 p.c. or less of oxygen in the inspired air [Campbell, 1935]. The partially protective effect of breathing carbon dioxide thus applies only to short acute experiments. That mammals cannot be acclimatized to live continuously under 10 p.c. or less of oxygen has recently been confirmed with a highly selected community of sulphur miners in the S. American Andes [McFarland, 1937].

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Recent types of face-tents for oxygen administration.

By J. Argyll Campbell

The box mask [1936] and face-tent [1937] have been modified to increase comfort. The shape has been altered slightly to widen range of vision and the oxygen inlet tube is placed horizontally inside the apparatus. The apparatus may be obtained in transparent celluloid acetate, or in aluminium (makers, Messrs Siebe Gorman).

Some patients have found the mask somewhat warm to the face, but this is easily corrected by exposing more of the skin of the arms to increase local rate of heat loss. Dr A. L. Barach (personal communication) has introduced a small injector on the oxygen inlet tube so that with 6 litres of oxygen a minute some air is drawn in and this increases the flow through the mask, which is reduced in size. This draught helps to remove all carbon dioxide and cools the face, but may be too rapid and disturb certain types of patients. There is also a whistle arrangement which gives warning when the oxygen tube is kinked.

Six litres of oxygen per minute give an alveolar oxygen pressure of 40–50 p.c. when the apparatus is properly adjusted, and excessive draughts are excluded from around the head of the patient. An additional advantage is that the apparatus may be used when the nasal passages are blocked, that is, when nasal methods (catheter, etc.) must be excluded.

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PROCEEDINGS
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PHYSIOLOGICAL SOCIETY
12 *March* 1938

The effect of androgenic substances on the rate of teat growth in the male guinea-pig. By A. C. Bottomley and S. J. Folley. (*National Institute for Research in Dairying, Shinfield*)

Administration of testosterone stimulates the growth of the mammary gland (Selye *et al.* 1936; Astwood *et al.* 1937]. Similar results with androstandiol and androstendione, but negative results with androsterone, were obtained by Nelson & Gallagher [1936]. The results of a study of the teat growth-promoting properties of various related androgenic substances are summarized in Table I. For each experiment

Table I

Injection	No. of animals in group	Ratio*	Ratio†	Difference
(*a*) 2 mg. androgen in 0·2 ml. sesame oil daily				
Sesame oil (control)	8	287	288	+ 1
Sesame oil	4‡	304	261	− 43
Androstendione	5	294	294	0
Androsterone	9	275	259	− 16
Dihydrotestosterone	5	308	310	+ 2
trans-Dehydroandrosterone	5	259	301	+ 42
17-Methyltestosterone	5	292	332	+ 40
Testosterone	4	285	332	+ 47
(*b*) 2 mg. androgen in 0·8 ml. sesame oil daily				
Sesame oil (control)	9	273	280	+ 7
Androstandiol	5	281	246	− 35
trans-Androstendiol	5	278	486	+208
trans-Androstendiol	5‡	283	473	+190

* $\frac{\text{Teat length (mm.)} \times 10^3}{\sqrt[3]{\text{Body weight (g.)}}}$ (initial). † $\frac{\text{Teat length (mm.)} \times 10^3}{\sqrt[3]{\text{Body weight (g.)}}}$ (28 days).

‡ The animals in these groups were castrated about 1 week before the commencement of injections.

a group of immature male guinea-pigs, each weighing about 170 g., was used, all animals receiving daily injections of 2 mg. androgen dissolved in 0·2 ml., or in the case of the less soluble diols 0·8 ml., sesame oil. Control groups were given equivalent injections of sesame oil alone. At intervals the lengths of the teats were measured to the nearest 0·1 mm. and the ratio

$$\frac{\text{Teat length}}{\sqrt[3]{\text{Body weight}}}$$

calculated. Table I contains the values of this ratio at the beginning of the experiment and after 28 days. The body weights of all animals increased steadily throughout the experiment.

The constancy of the ratio $\frac{\text{Teat length}}{\sqrt{\text{Body weight}}}$ for the control groups shows that, in the young male guinea-pig receiving injections of sesame oil, the rate of teat growth is isogonic [see Huxley, 1932], confirming the previous observations of Bottomley & Folley [1937]. This growth is apparently maintained by the testes, since castration causes the rate of teat growth to become negatively heterogonic. Of the androgens studied, the unsaturated ones, with the exception of androstendione, exhibit definite teat growth-promoting activity, *trans*-androstendiol being far and away the most active. Testosterone is about one-fourth as active as *trans*-androstendiol. In a previous communication [Bottomley & Folley, 1938] it was shown that all of the above androgens, except androsterone and *trans*-dehydroandrosterone, exert a marked "castration effect" on the young male guinea-pig, presumably by suppressing the gonadotropic activity of the hypophysis. It is therefore possible that androstendione and dihydrotestosterone possess slight teat growth-promoting properties, since in spite of, at any rate partial, suppression of testicular function they maintained isogonic teat growth.

We are grateful to Dr K. Miescher and Messrs Ciba, Ltd., for the androgens used in this work and to Mr J. McClemont for affording facilities for work with guinea-pigs.

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Androgenic activity of ovarian grafts. By Ruth Deanesly.
(*National Institute for Medical Research, Hampstead*)

Experiments and observations in recent years, summarized by Parkes [1937] and Lipschütz [1937], have supplied evidence of androgenic activity on the part of the ovary. Lipschütz [1932] found restoration of the accessory glands of a castrated male guinea-pig possessing a long-standing ovarian graft. Hill [1937*a*, *b*], in a remarkable series of experiments, has demonstrated that ovarian grafts in the

ears of castrated male mice may show androgenic activity which can be controlled by regulating the temperatures at which the mice are kept; at a cool temperature there is full restoration of the seminal vesicles in 2–3 months. Such growth could not be produced by œstrone or progesterone.

In view of Hill's results, similar grafts have been made in rats castrated when immature, in which the prostate weighs 16–20 mg. and the seminal vesicles 4–6 mg. Ovaries were transplanted into the ears either at the time of castration or 2–4 weeks later. Some of the rats were kept in heated animal rooms and others on a partially exposed balcony; they were killed 1–9 months after grafting. The following are the principal results so far obtained:

(1) Most of the functional grafts proved to be definitely androgenic whether or not the rats were kept at a low temperature.

(2) In the rats with grafts the prostates, with one exception, varied between 25 and 236 mg., but the seminal vesicles all tended to be small, commonly 20–50 mg. In one rat, however, the growth of the accessories was far greater, the prostate being of full adult size—910 mg.—while the seminal vesicles weighed 155 mg. Since the seminal vesicles are known to require more androgen than the prostate for their maintenance the disproportion in the weights is not surprising. In some rats the vesicular epithelium had remained inactive, while that of the prostate was restored and secreting. The apparent seminal vesicle growth in these rats was due to an increase in the smooth muscle [cf. Freud, 1933] produced by the œstrogenic secretion of the graft.

In general the weights of the accessory organs suggest that the androgenic activity of the graft is weak and is not due to testosterone.

(3) The degree of androgenic stimulation of the accessories could be correlated with the age, size and histological structure of the graft, e.g. prostates of over 100 mg. were only obtained when the grafts were more than 100 days old and weighed at least 27 mg. The androgenic activity seems to be essentially associated with luteinization of the theca interna of the follicles in the grafts.

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Changes in ease of extraction of myosotin-type proteins as a result of activity. By J. F. Danielli. (*Department of Physiology, University College, London*)

Saxl's [1907] observation, that part of the myosin in muscle can no longer be completely extracted after prolonged activity, has been confirmed and extended by Mirsky [1935], who has also found an analogous change in the myosin-like protein of sea-urchin eggs occurring after fertilization. In the present work the ratio R = insoluble protein : soluble protein has been used as an index of the amount of change. The solvent was that suggested by Mirsky (10 p.c. KCl, *p*H 7·2). For resting frog muscle $R = 0{\cdot}5$–$0{\cdot}8$; in iodoacetate rigour R varies considerably, from 1·4 to 3·0, but is always increased with rigour. For the leg muscle of *Carcinus* $R = 0{\cdot}25$ with resting muscle, 0·5 for iodoacetate rigour, and intermediate values with prolonged low frequency (5 per sec.) stimulation of the nerve. No change from the value of $R = 1{\cdot}2$ could be detected with the "non-medullated" leg nerve of *Maia*, after stimulation to exhaustion at 5 shocks per sec. Methods of breaking up the nerve prior to extraction were (*a*) grinding with solid CO_2, (*b*) grinding with KCl, (*c*) drying at −25° C. *in vacuo*, followed by grinding with or without solid CO_2.

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The catalytic effect of buffers on the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$. By V. H. Booth and F. J. W. Roughton. (*Physiological Laboratory, Cambridge*)

The rates of these reactions, in absence of carbonic anhydrase, are important to physiologists as a basis from which to work out the detailed kinetics of CO_2 transport within the body. They have usually been measured, at physiological *p*H's, in presence of buffers such as phosphate or cacodylate. Lately, however, we have shown [1937] that these buffers have some direct effect besides the instantaneous removal or supply of H^+ ions concerned in the ionization of carbonic acid ($H_2CO_3 \rightleftharpoons H^+ + HCO'_3$). Thus the overall rate of uptake of CO_2 by phosphate solutions at 0° C., v_u, is given by

$$v_u = 0{\cdot}0022\,[CO_2]\,\{1 + l_u\,[HPO''_4]\}, \qquad \ldots\ldots(1)$$

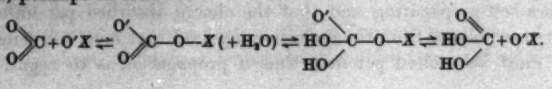
l_u being = 8. The figure 0·0022 is clearly the true velocity constant of the reaction $CO_2 + H_2O \rightarrow H_2CO_3$, whereas the term $l_u[HPO''_4]$ must represent a direct effect of the more negative constituent of the buffer. All other oxyacids which buffer in the range *p*H 6–9 showed similar effects, e.g. (i) cacodylate, arsenate, phosphite, chromate (l_u = 8–50), (ii) sulphite, selenite (l_u = 1000–5000), whereas salts of stronger acids all give much lower l_u values ($\gtrsim 1{\cdot}5$), which might be due to neutral salt action. The effects in class (ii) are clearly catalytic, selenite and sulphite being weak inorganic analogues of carbonic anhydrase. In class (i) it was first thought that the extra rate of CO_2 uptake might be in part due to direct non-catalytic combination of CO_2 with the buffer. The force of this idea, however, is much weakened by recent results on the output of CO_2 from mixtures of $NaHCO_3$ with various concentrations of buffer. In the case of phosphate, for example, we find that at 0° C. the overall output rate, v_o, is given by

$$v_o = 1{\cdot}7\,[H_2CO_3]\,\{1 + l_o[HPO''_4]\}. \qquad \text{.....(2)}$$

Special allowance was made for the effect of ionic activities in the experiments and calculations, a difficulty less met with in the CO_2 uptake work. The mean value for l_o, which is independent of *p*H (6·3–7·3) and of $[H_2PO'_4]$, = 8·7, which agrees well with the l_u value for phosphate, viz. 8·0. Similar agreement is found for the cacodylate ion, the l_u value being 8·8, whereas the l_o value is about 8·5. Cacodylic molecule concentration and *p*H (5·8–7·3) are again without effect. In mixtures of phosphate and cacodylate, the effects are additive. A preliminary experiment with selenite gave a high value (1700) of l_u, of the same order as l_o: selenite indeed has shown some remarkable features well worthy of further study as a possible model for the behaviour of carbonic anhydrase.

Since the buffer ions are seen to accelerate both phases of the reversible reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$ equally (within experimental error) their action must be in main catalytic. The output work has provided further evidence that the catalytic effects are due to the buffer itself, and not to some accompanying impurity.

Equations (1) and (2) show that CO_2 and H_2CO_3 are the substrates on which the catalyst acts. Whilst not ruling out a chain type of mechanism, we think it more likely that the action occurs in a series of intermediate stages, perhaps as follows:



As regards the first of these stages, Faurholt [1927] has shown that CO_2 combines readily in this way with the ions of alcohols, e.g. CH_3O', but to less than one-millionth the extent with the unionized alcohol. As he points out, the presence of the H^+ in the unionized molecule seems to inhibit the combination of CO_2: this may explain why cacodylic acid and $H_2PO'_4$ are apparently inactive as catalysts.

The present results should be borne in mind in any work with buffers containing CO_2 or HCO'_3 at physiological *p*H's, e.g. especially in investigating the *p*H activity curve of carbonic anhydrase.

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A capacitative theory of the local excitatory process in nerve. By Otto H. Schmitt[1]. (*Department of Physiology, University College, London*)

A considerable simplification of nerve excitation theory can be effected if one assumes that the local excitatory process consists primarily in a change of membrane capacitance rather than in a resistance breakdown. Some of the advantages of such a theory are: (1) no separate process of reconstitution need be postulated to explain recovery of irritability in the wake of an impulse as the membrane is entirely reversible; (2) no surface chemical reactions need be assumed to accompany the impulse and consequently energy consumption is low in accord with measurements of heat production; (3) partial excitation and decremental conduction are easily explained as the local process is not essentially all or nothing.

Since on this basis of a capacity shift the excitation of a local region is associated with the absorption of a fixed electrical charge per unit area rather than with a change of conductivity, this method also provides a simple explanation of the phenomena of accommodation without any additional assumptions concerning the properties of the membrane. Assuming that a certain local current I is required before the impulse becomes self-propagating and that the charge absorbed per unit area is q, it then follows from the definition $dq/dt \equiv I$ that a certain length of nerve must be excited per unit time if propagation is to occur. The

stimulating current must then rise at more than a certain minimal rate if propagation is to occur, an essential property of accommodation.

The behaviour of a nerve subjected to polarizing currents is also simply explained. The rise of threshold with anodal polarization is to be expected as the polarizing current subtracts from the stimulus. The fall in threshold with weak cathodal polarization represents addition of stimulus and polarizing current until the region just beneath the electrode becomes excited. The rise in threshold experienced with stronger cathodal polarization indicates that greater and greater lengths of the nerve are being kept in the excited state and that, for a propagated impulse, more and more remote regions have to be excited, and consequently stronger currents must be used.

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Late after potentials in nerve. By Otto H. Schmitt[1].
(*Department of Physiology, University College, London*)

If special precautions are taken to ensure a steady base line, certain phases of the nerve after potentials may be shown to persist for more than an hour. These potentials will be demonstrated.

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The spleen and the surface of red corpuscles. By J. G. Stephens.
(*Sir William Dunn School of Pathology, Oxford*)

Examination of the surface qualities of red corpuscles as expressed by their electrokinetic potentials measured with Brown and Broom electrophoresis cells in isotonic sucrose solution buffered at *p*H 7·4 reveals the following relationships to splenic function:

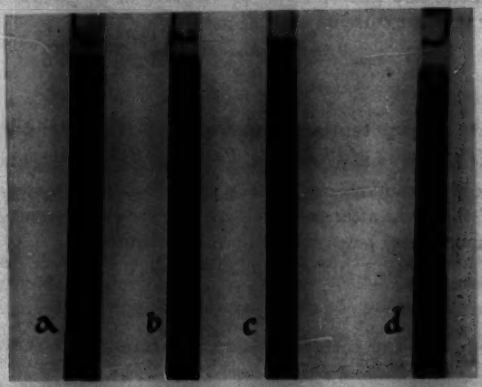
(1) Femoral vein blood of splenectomized dogs contains large numbers of red cells with electrophoretic speeds usually two-thirds of the normal value, $1{\cdot}86 \pm 0{\cdot}1\mu$/sec./V./cm., under the above standard conditions. These slow red cells and those of normal speed can be seen side by side simultaneously. They become marked in numbers about 3 weeks after splenectomy, and were not found in the 2 weeks immediately after removal of the spleen. After 3–4 months they have usually disappeared.

(2) Sedimentation rate examination of femoral vein blood of splenectomized dogs, citrated or heparinized, enables these red cells of low

electrokinetic potential to be floated off in a clearly marked stratum and thus isolated. Red cells withdrawn either from the upper or lower stratum fall into a sharply defined electrophoretic group, with speed the same as the slow and normal speed found when the blood is examined directly.

The upper zone consists predominantly of single non-aggregated red cells, another indication of their altered surface and the probable reason for their flotation. They have also a relatively lower density.

Similar but less marked stratification, with speed ratio 2:3, was seen in human blood in microcytic anæmia, and in unoperated anæmic dogs.



Stratified sedimentation after 2½ hr. of blood of splenectomized dogs. *a*, splenectomized 7 months; *b*, 6 months; *c*, 7 weeks; *d*, 17 days. 100 mm. columns. Blood: 3·8 p.c. sodium citrate = 4:1.

Stratification appears about the third week after splenectomy and is preceded by diffuseness of the upper limit of the sedimenting column.

(3) In blood samples, obtained by puncture of the exteriorized spleens of four dogs, the electrophoretic speed was more uniform than in the general circulation and equal to the highest speed found there. Not all splenic puncture samples showed this uniformity.

(4) Red cells obtained under ether anæsthesia from the spleen of a cat show a progressive increase in electrophoretic speed as the time of residence in the spleen becomes greater. The change is most marked if the corpuscles admitted to the spleen are abnormally slow, such as those found in pregnancy and other conditions of high sedimentation

rate. In these cases the speed of all the erythrocytes may be doubled after ½–1 hr. in the spleen. To a lesser extent the change occurs in the liver.

(5) The limit of speed reached by red cells from the spleen is approximately the same as the speed of fœtal red cells in the same animal. Unlike the maternal corpuscles the splenic and fœtal corpuscles are extremely uniform in speed.

(6) The sedimentation rate of splenic vein blood of cats and dogs (splenic artery clamped) shows a progressive decrease as successive samples are taken.

(7) Persistent contraction of the exteriorized spleen has been observed in (*a*) pregnancy, (*b*) lactation, (*c*) operative procedure, (*d*) aseptic necrosis of skin, (*e*) aseptic laparotomy and (*f*) peritonitis. It is now pointed out that increased blood sedimentation rate is a common factor in all these conditions. Persistent contraction of an exteriorized spleen has been observed to be related to increase in the blood sedimentation rate due to an oat-seed abscess. The hæmatocrit value did not decrease. Splenic contraction is therefore apparently a concomitant of altered plasma protein and ionic composition.

In explanation of these and other phenomena it is suggested that a primary function of the spleen is the repair of the red cell as regards its surface and electrolyte content, and that the repaired corpuscle is less vulnerable to osmotic damage and leakage during repeated ionic interchange of the respiratory cycle.

The spread of the local action potential in the single nerve fibre of the crab. By J. M. Ledingham and D. Scott, Jr. (*Department of Physiology, University College, London*)

Single fibres of the limb nerves of the shore crab (*Carcinus Maenas*) were investigated in the manner described by Hodgkin in his communication to the Society on 16 October 1937, and the results obtained with this preparation agreed entirely with his findings. The single nerve fibre, mounted on three fine platinum wire electrodes in a bath of oxygenated paraffin oil, showed an unpropagated monophasic potential when stimulated with subthreshold condenser discharges. This increased with the strength of the stimulus until threshold was reached and the normal conducted diphasic wave was seen rising from the maximum of the unpropagated potential. Increase of the stimulus

beyond threshold merely decreased the shock-spike interval until the conducted response masked the local effect.

The height of the local effect has been found to be proportional to the stimulus strength from zero to about 60 p.c. of threshold (see *A*, Fig. 1), but from here it starts increasing more and more rapidly until at threshold it is rising almost vertically. The portion of the local effect which increases linearly with the stimulus is due to catelectrotonus at the middle (the earthed) electrode and can be shown to increase with the

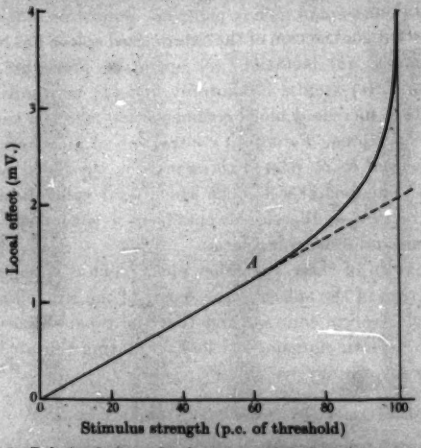


Fig. 1. Relation between local unpropagated monophasic potential and stimulus strength in single fibre of crab nerve.

stimulus in this manner indefinitely at the anode, or at both electrodes in a nerve that has ceased to conduct. In a nerve fibre that is conducting, with stimuli greater than 60 p.c. of threshold the observed potential is the sum of the electrotonic potential and the local action potential, the latter not being found when the fibre ceases to conduct.

When a fourth electrode is added and records are taken at different points on the nerve, so that the distance between the stimulating and recording leads can be increased from zero to 10 mm., it is found that both the electrotonus and the local action potential decrease to zero in a roughly exponential manner. Both fall to approximately one-third of their maximum value when the distance from the stimulating

cathode to the nearer recording electrode is 0·75 mm. In records where the conducted action potential spike was 22·7 mV., at threshold the local action potential was 3·5 mV. and the electrotonic potential 5·2 mV.

Apparatus for studying the asphyxiation of nerve at different temperatures. By H. SHAPIRO. (*Department of Physiology, University College, London*)

In frog nerve very low pressures of oxygen suffice to support respiration; consequently special precautions are required for purification of tank N_2 or H_2 if complete asphyxiation of nerve is to be attained. This is effected by eliminating all rubber connexions, and using vessels made entirely of glass. The nerve chambers contain platinum electrodes for stimulation, and calomel electrodes for recording injury potential and action current via small moist wooden blocks. The chambers are immersed in Dewar flasks at temperatures varying from 4 to 39° C. The time for disappearance of the action current varied from 55 min. at 39° to 12·5 hr. at 4°. The points fall along a logarithmic curve qualitatively paralleling the variation of respiration with temperature. It is suggested that in nerve there is a fixed supply of a reserve which is necessary to support conduction, and which is utilized during anaerobiosis at a rate dependent upon the kinetics of decomposition of a substance whose breakdown yields energy. The injury potential is far more independent of this than the action current, for a nerve can remain polarized for very long periods long after the action current has been abolished. Both action current and injury potential can be restored upon readmission of O_2. The after positivity is much more labile than either action current or injury potential, disappears quickly during asphyxiation, and is restored very slowly upon admission of O_2. Continuous tetanization of the nerve during anaerobiosis shortens the time for disappearance of the action current.

A rapid galvanometer system for myothermic experiments. By A. V. HILL. (*Department of Physiology, University College, London*)

The thermopiles recently constructed by Downing [Hill, 1937] are so rapid that no existing galvanometer does them justice: the galvanometer is either too slow or too insensitive, and valve amplification of E.M.F.'s of 2–10 μV. is unsatisfactory. Use, therefore, has been made of two galvanometers coupled by a differential arrangement of rectifying photocells [see Hill, 1934 *a*, *b*].

The current from the thermopile deflects the primary galvanometer. The movement of its beam of light produces a photoelectric current, which goes to a second galvanometer and causes a deflexion 15–20 times that of the first. The second galvanometer is of short period (0·06 sec.). Part of the photoelectric current is "fed back" [cf. Walton, 1935] to the primary galvanometer against the thermopile current, making it quicker: in this way a period of 0·2 sec. can be reduced to 0·07 sec. The arrangement has sufficient sensitivity with a deflexion time of considerably less than 0·1 sec. "Instantaneous" heating of a muscle on a quick thermopile may give 90 p.c. of full galvanometer reading in 0·05 sec.

Mechanical disturbances limit the useful amplification and so the degree of quickening possible. To minimize such disturbances the primary galvanometer, the photocell and the light are floated on a mercury bath damped with oil.

The great increase in speed, together with the use of a "protected" thermopile, makes possible a much closer analysis of the heat production of muscle in relation to work, shortening, stimulus, relaxation, release, etc.

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Experimental arterial hypertension in rabbits. Effect of section of renal nerves. By B. G. Maegraith and F. J. McLean. (*Sir William Dunn School of Pathology, Oxford*)

Average blood pressures recorded by the carotid loop method in rabbits were found to range from 68 to 106 mm. Hg. After the average pressures for each animal had been measured for a suitable period, 2 to 3 c.c. of a 1 p.c. suspension of Kieselguhr white in 1 p.c. gum arabic solution in physiological saline were injected into one renal artery. Eighteen animals surviving the injection for more than a week all showed a rise in arterial blood pressure of from 12 to 50 mm. Hg. In some animals the blood pressure rose at once after the injection and stayed up permanently. In others the initial rise was succeeded by a fall, followed in a few days by a further rise to a permanent high level. The high pressure persisted for periods of up to 5 months and did not return to the pre-operative average level without further operative interference (nephrectomy or section of the renal nerves).

In 11 animals showing raised blood pressures lasting from 3 weeks to over 5 months, the renal pedicles were stripped of nervous tissue from the aorta to the hilus of the kidney, and the adventitia of the artery was stripped off until the vessel appeared as a series of small aneurisms. In 9 of these animals the blood pressures fell to the original low level in 3 to 8 days after the operation. In the other 2 rabbits the pressures did not fall after the first stripping and further operation revealed intact renal nerves which had been missed previously. Section of these nerves produced a fall in blood pressure to the former average level of the animals. The blood pressures of all these animals have remained low since operation, i.e. for periods of up to 4 months.

Five rabbits had the left renal pedicle stripped and an injection of Kieselguhr suspension into the left artery performed at the same operation. The blood pressures of all these animals rose sharply immediately after the operation, but returned to the pre-operative level in 4 to 8 days and have remained low since, i.e. for as long as 4 months.

These effects on blood pressure obtained by stripping the renal pedicles of rabbits are not observed in dogs suffering from high blood pressure produced by constriction of the renal artery [Collins (1936), Page (1935)], or by X-ray of the kidneys (Page). It should be noted that Goldblatt *et al.* (1937) were unable to prevent or cure hypertension, produced by renal ischæmia in dogs, by bilateral excision of the lower four thoracic sympathetic ganglia and the thoracic portions of the splanchnic nerves.

Chemical changes. The blood urea nitrogen, determined by a modification of Conway's method [1933], rose sharply after the injection of the Kieselguhr suspension and returned to normal in 3 to 9 days, except in 2 animals in which the damage to the kidney was sufficient to cause death. The total blood nitrogen, measured by a micro-Kjeldahl technique, showed no significant variation after injection, and no significant variation was observed in the blood chloride.

After injection of the kidney, the output of urine per day decreased for a period of 3 to 10 days and then sometimes exceeded the former normal volume.

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